THE GENUS SALPA

A MONOGRAPH WITH FIFTY–SEVEN PLATES

BY

WILLIAM K. BROOKS, Ph. D., LL. D.

Professor in the Johns Hopkins University and Director of its Marine Laboratory

WITH A SUPPLEMENTARY PAPER BY

MAYNARD M. METCALF

Fellow of the Johns Hopkins University

CONTENTS.

PART I.—A GENERAL ACCOUNT OF THE LIFE HISTORY OF SALPA.

PART II.—THE SYSTEMATIC AFFINITY OF SALPA IN ITS RELATION TO THE CONDITIONS OF PRIMITIVE PELAGIC LIFE; THE PHYLOGENY OF THE TUNICATA; AND THE ANCESTRY OF THE CHORDATA.

PART III.—A CRITICAL DISCUSSION OF MY OWN OBSERVATIONS AND THOSE OF OTHER WRITERS, ON THE SEXUAL AND ASEXUAL DEVELOPMENT OF SALPA.

PART IV.—THE EYES AND SUBNEURAL GLAND OF SALPA.

By MAYNARD M. METCALF, *Fellow of the Johns Hopkins University.*

PART V.—EXPLANATION OF THE PLATES.

PART ONE.

A GENERAL ACCOUNT OF THE LIFE HISTORY OF SALPA.

CHAPTERS I, II, III, IV, AND V.

CHAPTER I.

INTRODUCTORY.

Most of the material for the researches on which this memoir is based was gathered and preserved at sea, at various points upon the eastern and western coast of the United States, by the vessels of the United States Fish Commission, under the direction of Marshall M'Donald, the Commissioner of Fisheries.

As the schooner Grampus was engaged during the summer of 1888 in work which gave, incidentally, an excellent opportunity for pelagic exploration, the commissioner invited me to avail myself of the advantage, and I owe to him the pleasure of a cruise in the Gulf Stream under conditions which were most favorable for studying its floating fauna.

I have also received from the commissioner, from time to time, collections of salpæ which have been gathered, under his direction, at various localities near our coast. These specimens have been preserved for microscopic research, according to approved methods, by Professor Wm. Libbey, Professor L. A. Lee and Mr. R. P. Bigelow, and I am indebted to these gentlemen for many valuable additions to my collection.

My thanks are also due to the National Academy of Sciences for a grant of money from the "Bache" fund, to aid me in the prosecution of this research; and to the Trustees of the Johns Hopkins University for the opportunity to study living salpæ at many points upon our coast, and for the provision which they have made, from the funds of the University, for the publication of this memoir.

As I enter upon the preparation of this account of a research which has been, up to this point, an unfailing source of pleasant interest, I am confronted by the disagreeable prospect of inevitable controversy; and all my pleasure is destroyed by the thought that nearly every one of my statements will contradict the published statements of some one among the numerous writers on the subject.

Scientific controversy is so unprofitable that I shall try to make it as subordinate as possible, that the reader may devote all his attention to the life-history of salpa without interruption at every point where my own observations confirm or contradict the statements of others.

The story of the life-history of salpa is most interesting, but it is complicated and difficult to tell in simple words, even after it has been stripped of all which is not essential, and I am sure that I shall promote the interest of my readers by strict adherence to the unity of my description.

I shall therefore give, in the first place, a continuous, uninterrupted account of the subject, and shall reserve the discussion of disputed points for later chapters.

I know that I have built upon a foundation which has been laid by others, and that many of the facts have been the common property of naturalists for many years, but truth and error are so closely bound together in the literature of the subject that it is not possible to give to each author the credit which is his due without entering into adverse criticism at the same time, and as the history of our knowledge of Salpa has been reviewed over and over again, it does not seem necessary to enter into it here.

The home of the salpæ is so remote from common observation that few persons except naturalists have a true conception of their scientific interest, or of their importance in the economy of the sea, although they occupy a prominent place in the mind of every one who has enjoyed the pleasure of studying the floating fauna of the open ocean, and is thus enabled to call up a mental image of pelagic life as a whole.

The older naturalists who explored the ocean in sailing vessels, when calms gave leisure for studying its wonders, found in them an unfailing subject for fascinating and delightful research. They are seldom found near the shore, however, and as they are so transparent that they are scarcely visible from the deck of a modern steamship, they are little more than a name to most of the naturalists of our own day; but to the student, even the name, salpa, itself calls up a long list of famous naturalists and explorers.

Among them is the friend of Linnæus, Peter Forskal, who lost his life while exploring Arabia in 1774. His description of the animals which he had observed in his journey to the East was edited and published by his fellow-explorer, Niebuhr; and in it eleven forms of salpæ, which he had observed and studied in his voyage through the Mediter-

ranean, are so faithfully described that most of them can still be identified, and continue to bear the names which he gave them. He also proposes for them the generic name salpa, and gives the first satisfactory definition of them.

We think also of Chamisso, who, in 1814, left his romances and his poetry to go round the world with Kotzebue, and who found in the life-history of salpa a story more strange and wonderful than that of Peter Schlemihl; of Quoy and Gaimard, who, after many years of wandering among the islands of the Pacific with Freycinet and with Dumont d'Urville, enriched our literature with the zoological treasures of the "Voyage autour du monde" and the "Voyage de la corvette l'Astrolabe."

These and many other famous names are associated with the zoology of salpa, but the history of the literature of the subject has been reviewed so many times that we cannot dwell upon it, and the reader may refer to the bibliography which is given by Traustedt in his Spolia Atlantica, (Vidensk. Selsk. Skr., 6th Raekke 2, viii, Kjobenhavn, 1885, pp. 341–346,) or to the historical sketch and bibliography which are given by Herdman in the introductory chapters to his various reports on the Tunicata of the Challenger Expedition, (Report upon the Tunicata collected during the Voyage of H. M. S. Challenger during the years 1873-76).

I shall not duplicate these lists, but since there are a few papers to which I shall have to make frequent references, I give them here in a numbered series, and I shall refer to them hereafter by number.

1. LEUCKART, Dr. RUDOLF. Zur Anatomie und Entwicklungsgeschichte der Tunicaten. Zoologische Untersuchungen 2. Giessen, 1854.

2. SALENSKY, Dr. W. Ueber die embryonale Entwicklungsgeschichte der Salpen. Zeit. f. wiss. Zool. XXVII, pp. 179–237. Plates XIV–XVI.

3. SALENSKY, Prof. W. Ueber die Entwicklung der Hoden und über den Generationswechsel der Salpen. Zeit. f. wiss. Zool. XXX, Supplement, pp. 375–393. Taf. XIII. 1878.

4. BARROIS, Dr. J. Mémoire sur les membranes embryonnaires des Salpes. Journ. de l'Anat. et de la Phys., pp. 455–498. Plates XVII and XVIII. 1881.

5. SALENSKY, Prof. W. Neue Untersuchungen über die embryonale Entwicklung der Salpen. Mittheilungen aus der Zoologischen Station zu Neapel, I, pp. 90–171 and 327–402. Plates VI–XVII and XXII–XXVII. 1882.

6. KOWALEVSKY, A., and BARROIS, J. Materiaux pour servir a l'histoire de l'Anchinie. Journ. Anat. Phys. XIX, 1883, pp. 1–23. Plates I–III.

7. ULJANIN, Dr. BASILIUS. Die Arten der Gattung Doliolum im Golfe von Neapel. Fauna und Flora des Golfes von Neapel, X, 1–140, with twelve plates. 1884.

8. TRAUSTEDT, M. P. A. Spolia Atlantica. Bidrag til Kundskab von Salperne. Mem. Acad. Royal Copenhagen, 6, II, 8. 1885. Ten plates.

9. SEELIGER, Dr. OSWALD.   Die Entwicklungsgeschichte der socialen Ascidien. Jenaischen Zeitschr. XVIII, N. Z. XI, 1–145.   Taf. I–VIII.   1885.

10. BARROIS, Dr. JULES.   Recherches sur le cycle génétique et le bourgeonnement de l'Anchinie.   Journ. Anat. Phys. XXI, 1885, pp. 193–267.   Plates VIII–XII.

11. SEELIGER, Dr. OSWALD.   Die Knospung der Salpen.   Jenaische Zeitschr. XIX, 573–677.   Plates X–XIX.   1886.

12. BROOKS, W. K.   The Anatomy and Development of the Salpa-Chain.   Studies Biol. Lab. Johns Hopkins Univ. 1886, pp. 451–487.   Plates XXVIII, XXIX.

13. SEELIGER, Dr. OSWALD.   Die Entstehung des Generationswechsels der Salpen. Jenaische Zeitschr. XXII, 398–414.   1888.

14. HERDMAN, Dr. W. A.   Report upon the Tunicata collected during the Voyage of H. M. S. Challenger during the Years 1873–76.   Part III.   Report on the Scientific Results of the Voyage of H. M. S. Challenger.   Vol. XXVII, Part LXXVI.   1888.

15. SEELIGER, OSWALD.   Zur Entwicklungsgeschichte der Pyrosoma.   Jenaische Zeitschr., pp. 595–658.   Plates XXX–XXXVII.   1889.

16. DAVIDOFF, Dr. M. v.   Unters. zur Ent. der Distaplia magnilarva Della Valle. Mittheilungen aus der Zool. Station zu Neapel, IX, 113–178 and 533–562.   Plates V, VI and XVIII–XXIV.   1889–91.

17. SALENSKY, Prof. W.   Beiträge zur Embryonalentwickelung der Pyrosoma. Zool. Jahrbücher, Band IV, 424–478.   Taf. XXVI–XXVIII; Band V, 1–98.   Taf. I–VIII. 1891.

18. D'KOROTNEF, Dr. ALEXIS.   La Dolchinia mirabilis.   Mittheilungen aus der Zool. Station zu Neapel, X, 2, 1891, pp. 187–205.

Salpa is a transparent, swimming Tunicate, and its body, Plates III and IV, is, in effect, an enormous pharynx which swims through the water, gulping in great mouthfuls at each contraction of its muscles.

In shape it is subcylindrical, and its body may be compared to a barrel open at both ends, so that water flows through it without obstruction.   The mouth, r, occupies the anterior end of the barrel, and the lips are infolded in such a way that they act as valves which permit the water to enter between them while they prevent it from escaping, while, at the opposite end of the body, the atrial aperture, g^v, affords an exit for it.   Practically the chamber of the barrel is uninterrupted from one opening to the other, for while it is divided, morphologically, into the pharynx, c, and the atrium, g''', these two chambers are separated from each other only by the rod-like "gill," o, which traverses the cavity on the middle line.   The "gill" is so narrow that it offers little obstruction to the water, and there is a free passage on each side of it.

The body is encircled, more or less completely, by muscles which are placed like barrel-hoops, so that their contraction empties the barrel and drives the water out through the atrial aperture, and thus propels the

floating animal through the water in the opposite direction. Around the mouth the muscles are so placed that they close the lips and prevent the water from escaping in this direction when the barrel contracts.

The body is inclosed in a thick outer mantle, Plates I and II, which by its elasticity antagonizes the muscles, and expands the barrel and draws in a fresh mouthful of water. In most species the atrial aperture, Plate I, Fig. 3, is encircled by sphincter muscles which constrict it and prevent the water from entering during expansion, so that the animal moves forwards, by jerks, along a column of water which passes through its body.

The food of salpa consists of radiolarians, diatomes, and other micro-organisms which float in the water, and as these lodge on the inner surface of the barrel they are gathered up and swept through the œsophagus into the stomach in a way which will be described soon. The supply of this food is unlimited, and salpæ are often found swarming at the surface of the ocean in number beyond description.

In size they range from 6 mm. or about one-fourth of an inch, the average length of the aggregated form of Salpa democratica, Plate II, Fig. 1, to about 20 cm. or eight inches, the length of a large specimen of the solitary form of Salpa costata, Plate IV, Fig. 4.

Although they are met with in the greatest variety and abundance in the warmer parts of the ocean, they are by no means confined to the tropics, and they have been found in great numbers north of Norway and Scotland and south of Cape Horn and the most southern points of Australia and New Zealand.

They are abundant only after the water has been for some time undisturbed by winds; and as prolonged calms are most frequent in warm seas, these waters are most favorable for the development of these animals, which multiply with astonishing rapidity. The smaller species are often so abundant that for hundreds of miles any bucketful of water dipped up at random will be found to contain hundreds of them. In such places collecting with the surface-net becomes impracticable, for almost as soon as the net is dropped into the water it becomes choked with a mass of salpæ so that nothing more can enter it.

A drop from an organic infusion swarming with infusoria, seen under a low power of the microscope, bears some resemblance to the surface of the ocean when salpæ are abundant, except that the water is not turbid like the infusion, but beautifully clear and transparent.

No one who has not seen these animals under favorable conditions can form any conception of the amount of animal life which pure sea-water is able to support; and the salpæ are able to multiply with great rapidity, both sexually and asexually, in order to avail themselves quickly of favorable conditions.

Each species of salpa has two generations in its life-cycle, known as the solitary generation and the aggregated generation.

The solitary salpa is born from an egg which is carried within the body of the aggregated salpa, whose blood nourishes the embryo during its development by means of a nutritive placenta, Plate III, Fig. 4, and Plate XXXV.

The aggregated salpæ are produced asexually from the body of the solitary salpa.

Plate II shows the solitary form of Salpa democratica, and Plate XLIII, Fig. 1, shows the aggregated form of the same species. In Plate I, the solitary form of Salpa pinnata is shown in dorsal view in Fig. 5, and in ventral view in Fig. 6. The aggregated community of the same species is shown in Fig. 2, and one of the members of the community in Fig. 3. Figs. 4 and 7 show the dorsal and ventral aspects of the solitary form of Salpa chamissonis, and the aggregated cormus is shown in Plate XLI, Fig. 9, and one of its members in Plate VIII, Fig. 6. The solitary form of Salpa cordiformis is shown in Plate IV, Figs. 3 and 4, and a part of a "chain" of the same species in Plate IV, Fig. 6. Plate IV, Fig. 7, is the embryo of the solitary form of Salpa scutigera, and Plate IV, Fig. 1, part of its "chain," and so on.

In most species each aggregated salpa carries only one egg, so that the solitary generation consists of only one individual; but in all the species the aggregated generation consists of many hundred individuals, and there is reason to believe that it has no fixed limit, but that the solitary salpa may continue to produce aggregated salpæ for an indefinite time and in unlimited numbers.

The aggregated salpæ are born in sets or cormi. In the chain-like cormi the number of individuals is usually more than a hundred, but in circular cormi it is very much smaller, and each cormus of Salpa pinnata, Plate I, Fig. 2, contains only eight or nine.

In a few species, each aggregated salpa contains more than one egg, but the number is small, and seems to be fixed and constant for the species. In Salpa cordiformis, for example, there are five eggs, and each chain-salpa gives birth to five embryos, Plate III, Figs. 2 and 3, *em*. Salpa hexagona, Plate X, Fig. 10, also produces five embryos.

The solitary salpa of each species differs from the aggregated form of the same species in many of the details of its structure, and in many cases the difference is very considerable, but the fundamental plan is the same for all.

The specific characteristics of each solitary salpa are quite different from those of its corresponding aggregated form, so that there is no way of deciding what specimens of the two forms belong together, except by actually rearing them, or by the discovery in the body of a given chain-salpa of an embryo sufficiently advanced in development to exhibit its adult characteristics; or else by finding them associated in great numbers free from admixture with other species. The two forms, when mature, are nearly equal in size, and this is sometimes an aid in identifying them.

The divergent modification which has produced the various species has affected the two forms of each species in different ways, but to about the same amount, so that we cannot say that the solitary salpæ of different species are less or more differentiated than the aggregated salpæ.

This fact indicates clearly that the separation of the life-cycle into the two generations took place before the species diverged from their common ancestor.

The general features of the structure of a solitary salpa are well shown in the longitudinal vertical section of an embryo of Salpa pinnata in Plate XXXV, and in the horizontal sections in Plate XIX, and in the embryos of this species in Plate XLI, Figs. 1, 2, 3 and 4, and in the embryo of Salpa hexagona in Plate III, Fig. 4.

The structure of the aggregated salpa is shown in Plate VIII, where Fig. 1 shows two young aggregated salpæ of Salpa pinnata, and Fig. 2 two of Salpa cylindrica.

As the figure of the solitary Salpa africana in Plate IV, Fig. 2, shows, the body is subcylindrical in shape, and the two orifices, the mouth, r, and the atrial aperture, g^v, which are usually close together in sessile tunicates, are widely separated and are nearly or quite at opposite ends of the body.

In the solitary salpæ of all species, and in nearly all the aggregated salpæ, the mouth is terminal, or at one end of the cylinder. In a few aggregated forms, Salpa cordiformis, for example, Plate IV, Fig. 6, it is not at the extreme end, but on the outer surface.

Figure 8 of Plate XLV is a vertical section through the oral region of the embryo of Salpa pinnata just before the mouth is formed. The figure

shows that the upper lip is rounded and protuberant and thick, while the lower lip is very thin. In mature specimens this thin lower lip folds inwards so that the thickened upper lip shuts closely upon it when the mouth is closed. The flexible edge of the lower lip acts as a valve, for it is depressed by the inflowing current of water, while back pressure folds it up against the upper lip and closes the mouth. In many species there are two short muscles on the upper surface of the upper lip to open it. They lie near the middle line, and they are parallel or nearly parallel to each other. They are shown in Plate II.

The muscles for closing the mouth are much more complicated, as many of the figures show: Plate IV, Fig. 2, for example. Their arrangement in the solitary salpa is usually different from that in the aggregated form, and they also vary according to the species, but in most cases some approximation to the arrangement shown in Plate XLI, Figs. 3 and 5, can be made out, although the oral musculature is seldom as well developed as it is in these figures, which represent solitary embryos of Salpa pinnata. The mouth is not yet open in these embryos, but r marks its position. A bridle-like muscle arises from about the middle of the second body-muscle,—the body-muscle which crosses the upper surface of the body just behind the ganglion, s,—and runs across the oral end on what is to become the angle of the lower lip, and, crossing the middle line, unites to the middle of the second body-muscle on the opposite side. At its lower end this body-muscle joins a muscle which runs upwards and forwards to the angle of the mouth, where it divides into three branches, two superior ones which cross the middle line on the convex surface of the upper lip, and one inferior one which crosses the middle line just below the mouth. These muscles are shown in section in Plate XLV, Fig. 8. In adult salpæ the oral muscles are usually quite distinct from the body muscles in their anatomical relations, and very much smaller; but these embryos show that they belong to the system of body-muscles, or perhaps it is more near the truth to say that the body-muscles of salpa are modified oral and atrial sphincters.

The body-muscles exhibit the greatest diversity of arrangement, as may be seen by comparing the more divergent forms, such as Salpa hexagona, Plate III, Fig. 4; Salpa scutigera, Plate IV, Fig. 7, and Salpa costata, Fig. 4.

It is hard to say what number of muscle-bands is most characteristic; nine seems to be more common than any other number, but in some species, Salpa scutigera for example, they are reduced to four, while in

Salpa costata there are twenty. They may be continuous, as in Salpa pinnata, Plate XLI, Fig. 5, or interrupted as in Salpa costata, Plate IV, Fig. 4. They may be independent as in the last species, or united in sets as in Salpa democratica, Plate I. They may be restricted to the dorsal surface of the body, as in Salpa africana, Plate IV, Fig. 2, or they may be completely closed rings passing entirely around the body like the hoops around a barrel, as in the solitary form of Salpa democratica, Plate II.

Leuckart says (I, p. 15) that while the gap may be so short that it is not visible without a microscope, they are always interrupted on the middle line; but I am at a loss to find the basis for this statement, for the fibers may be traced across the middle line, without any break, in many species. He also says, p. 16, that as they never cross the ventral middle line they are never complete rings, and this statement has been repeated over and over again until it has found its way into all the text-books and scientific memoirs, notwithstanding the fact that most of them are completely closed, dorsally and ventrally, in the most familiar and abundant species, the solitary Salpa democratica, Plate II.

This error has been most persistent, and it has been made the basis of the fundamental definition of the whole Salpa family, for which Claus has proposed the name Desmomyaria, and Herdman the name Hemimyaria, to distinguish them from the Doliolums, for which Gegenbaur has proposed the family name Cyclomyaria.

Even if this difference between Salpa and Doliolum were absolute, the selection of a characteristic so very variable as the form of the loco-motor muscles as a basis for fundamental classification would be most unwise; and, as a matter of fact, some of the muscle-bands of Doliolum are incomplete, and some of them, in at least one species of Salpa, complete. In the first generation or "Amme" of Doliolum the seventh body-muscle is incomplete dorsally, and in the median "Pflegethiere" it is incomplete ventrally, while the lateral buds or "Ernährungthiere" depart very widely from the cyclomyarian type.

So far as I am aware, Traustedt is the only modern writer on Salpa who has described the muscle-bands of Salpa democratica as complete circles. In his description of this species, p. 365, and also in his descrip-tion of the variety flagellifera, p. 369, he states the facts correctly, but while his draughtsman, Cordts, has figured Salpa democratica correctly, Plate II, Figs. 25 and 26, he has followed tradition in his figure of Salpa flagelli-fera, Plate I, Fig. 12, rather than nature and Traustedt, and has drawn

all the muscle-bands as interrupted ventrally; so hard is it to correct established error.

The atrial aperture is sometimes valvular, as, for example, in the aggregated form of Salpa cordiformis, Plate III, Fig. 2, g^v, but it is usually a simple opening, circular or nearly so, without valves. Its mode of origin in the embryo of Salpa pinnata is shown in Plate XVII, Figs. 6 and 7. Quite frequently it is produced into a projecting funnel, as in Salpa costata, Plate IV, Fig. 4, g^v. It may be terminal at the posterior end of the body, as in Salpa pinnata, Plate I, Figs. 1, 3, 5 and 6, or it may be on the upper surface of the body at some distance from the posterior end, as in Salpa democratica, Plate II and Plate XLIII, Fig. 1. There is little uniformity in its position. In Salpa democratica it is on the upper surface in both the solitary form and the aggregated form. In Salpa pinnata it is terminal in both forms, as it is also in Salpa costata. In the solitary form of Salpa cylindrica, Plate III, Fig. 5, it is terminal, while it is on the upper surface of the aggregated form, Fig. 6, while the reverse of this is the case in Salpa hexagona, Figs. 4 and 1.

The atrial aperture is usually encircled by sphincter muscles, as in Salpa cylindrica, Plate III, Fig. 5. These muscles are usually a continuation of the series of body-muscles, which gradually become narrow and crowded together as they approach the cloacal aperture. In these cases the atrial sphincters are often complete rings. In a few species they have a more complicated arrangement, as in Salpa costata, Plate IV, Fig. 4 and Plate VIII, Fig. 4, where they branch out in a dendritic manner from two lateral longitudinal trunks. The chain-form of Salpa cordiformis, Plate IV, Fig. 6, has the same arrangement in a rudimentary or slightly developed condition. Often there is a lack of bilateral symmetry in the posterior body-muscles of aggregated salpæ. In the aggregated form of Salpa costata, Plate VIII, Fig. 4, the branches of one lateral trunk are very slightly developed, while those from the other almost encircle the atrial funnel.

The last body-muscle of the aggregated form of Salpa cordiformis, Plate III, Fig. 2, is forked on the right side but not on the left.

As Leuckart pointed out long ago (I, p. 6), the mouth and the atrial aperture are very much nearer each other in the young salpa than they are in the adult, as examination of my figures will show.

Figure 2 of Plate XLI shows a young embryo of Salpa pinnata with the point where the mouth is to be formed marked r, and the position of the atrial aperture marked g^v. It will be seen that the interval between

these two points is considerably less than a fourth of the circumference of the body. In Fig. 3, and in Plate XXXV, they are a little further apart; still further apart in Fig. 5, while in the adult, Plate I, Fig. 4, they are at opposite ends of the long axis of the body. The history is the same in the aggregated form, as is shown by the series of figures of the aggregated Salpa pinnata in Plate VII, Figs. 4 and 5; Plate VIII, Fig. 1, and Plate I, Fig. 3.

The test of salpa has never received the attention it merits. Most of the figures and descriptions of the species are from preserved specimens, and as the muscles are made opaque and distinct by alcohol, while the test remains transparent, and usually becomes more or less swollen and indefinite by the action of preserving fluids, the published descriptions contain very few references to the fact that in many species the surface of the body is delicately sculptured and is marked by ridges and serrations.

In this particular most of the figures are untrue to nature, and as I have attempted to show in my figures the structure of the test, many of my illustrations of familiar species are so different from those which usually pass as correct drawings that I feel called upon to explain the discrepancy.

In many species the test is divided by longitudinal ridges or keels into thick and thin portions, and the ridges are sometimes simple and sometimes serrated. Their physiological function is undoubtedly to give strength and stiffness to the test, that it may antagonize the muscles more effectively, and restore the shape of the body after contraction.

While the different species which possess these ridges exhibit considerable variation, there is a general plan which can always be recognized. On the middle line of the dorsal surface the test is thin, and when the muscles are contracted, as they usually are in preserved specimens, this thin area, Plate III, Figs. 1, 2, 3 and 5, forms a deep longitudinal furrow, bounded on each side by the prominent, keel-like edge of the thickened portion of the test. Along each side of the body there is usually a dorso-lateral keel, and the thickened portion of the test often forms a prominent "wing" on each side of the body between this keel and the one nearer the middle line, as is shown in Fig. 5 and on the left side of Fig. 1. On the sides of the body the test is thin, and sometimes strengthened by a lateral ridge on each side. On the ventral surface the test is thick, and is bounded at the sides by two ventro-lateral keels, Fig. 7, while there is often a median keel on the middle line of the ventral surface. I have found these ridges in the solitary form of Salpa demo-

cratica, Plate II; in both the aggregated form of Salpa hexagona, Plate III, Fig. 1, and the solitary form; in both the aggregated form of Salpa cordiformis, Plate III, Figs. 2 and 3, and the solitary form, Plate IV, Fig. 5; in the solitary form of Salpa cylindrica, Plate III, Figs. 5 and 7; in the aggregated form of Salpa runcinata, Plate XLIII, Fig. 2, and in the solitary form, Plate XLIII, Fig. 3; and in the solitary form of Salpa africana, Plate IV, Fig. 2, and the careful study of living specimens will undoubtedly show that they exist in most of the species.

The aggregated form of Salpa runcinata is shown in Plate XLIII, Fig. 3. It has the two serrated ridges on the upper surface, running from the posterior end of the body to the region of the mouth, and it has three more ridges on the lower surface. The lower surface of the solitary form of this species, Plate XLIII, Fig. 2, is so highly ornamented that I should be almost disposed to regard it as a new species, if it did not agree in all other respects with the published descriptions, and had I not found markings of the same sort in so many other species. The arrangement of the serrated ridges in this species is so much like those of Herdman's Salpa echinata that I am almost disposed to believe that this species is a Salpa runcinata. Except for a slight difference in the muscles its internal structure is like that of Salpa runcinata, and I have found great variation in the arrangement of the muscles in all the species of the runcinata group.

I have not found the ridges in my specimens of the aggregated form of Salpa cylindrica, Plate III, Fig. 6, but they are well developed in the solitary form and, except that they are not serrated, they are almost exactly as they are in Salpa runcinata. They are shown on the upper surface in Fig. 5, and on the lower surface in Fig. 7.

The digestive organs of Salpa consist of the pharynx, c, which opens externally through the mouth, r, and communicates through the œsophagus, Plate VIII, Fig. 2, q, with the stomach, p, from which the intestine, p', runs to the anal orifice, p'', by which the intestine opens into the atrium, g'''. In most species of Salpa the digestive organs, with their accessory glands, and in the aggregated form the testis also, are bound together into a compact "nucleus," Plate IV, Fig. 2, which is so solid and opaque in the adult that its structure can be studied only by sections. The arrangement of the digestive organs is essentially like Fig. 2 of Plate VIII, however, and those of the solitary salpa are usually like those of the aggregated salpa.

The œsophagus, beginning a little to the right of the base of the "gill," o, runs towards the upper surface of the body to open into the stomach which communicates with one or two blind diverticula. The intestine arises on the lower side of the stomach, and, describing a curve, passes to the left of the œsophagus, and opens into the median atrium at or a little to the left of the middle line above the base of the "gill."

In the species of the Pinnata group there is no compact "nucleus," and the anatomy of the digestive organs is quite different from that of the other species, and there is great difference between those of the solitary and those of the aggregated form.

In the solitary Salpa pinnata, Plate I, Fig. 5, the solitary Salpa chamissonis, Plate I, Fig. 4, the solitary Salpa affinis, and probably the solitary Salpa dolichosema, the intestine, Plate XXXV, p, runs through the gill, and the anus is at the extreme anterior end of the median atrium. In the aggregated Salpa affinis and Salpa chamissonis, Plate VIII, Fig. 6, the digestive tract is coiled upon itself much as it is in ordinary salpæ, except that the coils are not bound together into a compact nucleus; but in Salpa pinnata the intestine of the aggregated form is nearly ventral in position, Plate VIII, Fig. 1, p, and the anus is far forward and close to, but on the left of the middle line. The history of its development in this species shows that when it first makes its appearance the digestive tract of the aggregated form is like that of ordinary salpæ, and that it has the position which is shown in Salpa cylindrica, in Fig. 2, with the intestine, p, crossing to the left of the œsophagus, q, and running towards the dorsal surface to open into the cloaca above the base of the gill. As the aggregated Salpa pinnata grows, the intestine and anus move downwards along the left side of the body, and at the stage shown in Plate VII, Fig. 5, the œsophagus and intestine lie at the same level. The left-hand salpa in this figure has its right side towards the observer, and the œsophagus, q, is seen to run from the pharynx to the stomach, p', while on the left side of the right-hand salpa the intestine, p, and anus, p'', are shown in almost exactly the same position. At an older stage, Plate VIII, Fig. 1, the intestine, p, seems in surface view to be on the middle line, but sections, Plate XXXVIII, Figs. 52 and 80, p, show that it is actually to the left of the middle line, although very close to it. In view of its history in Salpa pinnata, I think there can be no doubt that the primitive position of the digestive tract in all species of salpa is like that which is shown in Plate VIII, Fig. 2, and that, in this particular, the pinnata-like species have undergone secondary modification.

The microscopic organisms which form the food of salpa are gathered up and conveyed into the stomach by means of an apparatus which has been described so frequently that only a very brief account of it need be given, although its prominence during the development of salpa demands some acquaintance with it in order to render the account of the life-history intelligible.

On the middle line of the ventral surface of the pharynx there is a longitudinal furrow, Plate XLV, Fig. 5, D, bounded by two thickened borders. This structure is the endostyle. It is shown at *end* in Plate IV, Fig. 2, and in many of the other figures. In the adult some of the cells of its walls are glandular, secreting an adhesive substance, while other cells carry cilia, which are so placed as to slide the adhesive excretion along the endostylic furrow to the anterior end of the body, where the endostyle ends between two *ciliated bands*, *cb*, one of which lies on each side of the inner surface of the pharynx just inside the mouth. Each ciliated band consists of two parallel rows of cilia close together, and by their activity the adhesive matter from the endostyle is drawn upwards along the sides of the pharynx, in fine threads, which stick to and entangle all the organisms which touch them as they are swept in by the current of water which passes through the pharynx.

The pharynx, Plate IV, Fig. 2, *c*, and the atrium, *g'''*, are in free communication with each other, as already noted, except on the middle line, where they are separated from each other by the "gill," *o*. In a median longitudinal section, like the one shown in Plate XXXV, the outline of each chamber is well marked. In a young embryo the "gill" is nearly horizontal, and the atrium is above the pharynx, but as the animal grows up the "gill" becomes more and more inclined, until in the adult, Plate IV, Fig. 2, its posterior end approaches the ventral side of the body, and the pharynx and atrium lie end to end; the former extending farthest backwards on the ventral middle line, while the latter extends farthest forwards on the dorsal middle line. On each side of the "gill" the two chambers communicate with each other, and I have not been able to find in the adult any indication whatever, in their side walls, of the line where the one chamber ends and the other begins. In front of the ganglion, *s*, the pharynx occupies the whole cavity, as does the atrium behind the nucleus, *nu*, and the imaginary line where they meet is probably inclined, like the "gill," so that the cavity of the pharynx diminishes in size as we pass backwards, while the atrium increases at the same rate. In Salpa costata, where the nucleus is a considerable distance in front of the atrial aperture, the tubular portion of the atrium is very long.

The "gill" of salpa is a respiratory organ, and a true gill in the physiological sense, but it is not homologous with the structures which in ordinary tunicates are called gills. This name is usually applied to the clefts or slits in the sides of the pharynx by which this communicates with the lateral atria or peribranchial spaces.

In salpa, as the life-history shows, there is one enormous gill-slit on each side of the body, and the "gill" is simply the portion of the body cavity which lies on the middle line between the pharynx in front and below, and the atrium above and behind, while its sides are the inner edges of the two gill-slits. It therefore corresponds, as Herdman (p. 56) has pointed out, to the structure which in ordinary ascidians he has called the dorsal lamina. I shall give further on my reasons for believing that the gill-slit on each side of the pharynx of salpa has actually arisen by the coalescence of all the gill-slits of an ancestor which had a pharynx like that of ordinary ascidians. At present, however, it has lost all traces of this history, even in the embryo. Herdman says that in Salpa bicaudata there are traces of stigmata along the sides of the gill, but I have made sections through the gill of the embryo of Salpa scutigera, at the stage shown in Plate IV, Fig. 7, and find no trace of stigmata, and according to Traustedt, bicaudata is a synonym for scutigera.

The central nervous system of salpa is a compact subspherical ganglion, placed midway between the mouth and the atrial aperture, on the dorsal surface, in the position which it occupies in the sessile tunicates, in which these two apertures are close together; and it is so different from the elongated tubular nervous system of the primitive chordate type, as shown, for example, by the larvæ of the ascidians, that we are forced to believe that it has been affected by the same influences as those which have led to its centralization in the sessile tunicata.

In all respects the general plan of the structure of salpa is fundamentally identical with that of the ordinary tunicates, and the differences are differences of detail. The atrial aperture, instead of being near the mouth, as it is in ordinary tunicates, is widely removed from it, as it is also in Doliolum and Pyrosoma; and the atrium, instead of being wrapped around the pharynx as it is in the ordinary tunicates and, to a less degree, in Pyrosoma and Doliolum also, is placed end to end with it, but there is no reason whatever for questioning the strict homology of the atrium of Salpa with that of the other tunicates.

This homology has been questioned by several recent writers on the development of Salpa and Doliolum, but I shall show that the history of

the development of both the solitary and the aggregated salpa proves that there is no basis for this opinion.

On the contrary, the facts of embryology are in perfect accord with the teachings of comparative anatomy, and can be explained in only one way, that is, by unqualified acceptance of the view of the older writers, that the atrium of Salpa is the same chamber as the atrium of the ordinary tunicates.

This short sketch is not intended to be an account of the structure of salpa, and it is given only to enable the reader to follow the account of the life-history which follows.

CHAPTER II.

THE DEVELOPMENT OF THE SOLITARY SALPA FROM THE EGG.

SECTION 1.—*An Outline of the History of the Egg.*

The history of the eggs of Salpa before they are fertilized is so inti-
mately bound up with the history of the process of asexual multiplication,
that it is difficult to describe the one without continual reference to the
other, and I shall therefore leave the detailed discussion of the origin and
homologies of the germ cells for a later chapter, after the process of
asexual multiplication has been described. It will, however, be best to
speak briefly of the early history of the eggs before we enter upon the
description of the process by which the ripe fertilized egg becomes con-
verted into a salpa embryo.

The germ cells are definitely set apart for reproduction at such an
early stage, that our account of the embryology of salpa must begin with
the embryo of the preceding generation; for very early in its life, while it
is still an embryo, we find in its body cavity a sharply defined mass of
cells which the study of older specimens shows to be the germ of the
reproductive organs. It is shown at *n* in Plate XXXV. At the earliest
stage in which it can be identified, it lies in the body cavity of the
embryo on the middle line of the ventral surface, and it marks the spot
where the proliferous stolon is afterwards to be developed. As this latter
is gradually formed the germinal mass is folded into it, in a way which
is made clear by the successive stages shown in Plate XX, Fig. 6, Fig. 5,
Fig. 7; Plate XXXV, *n*, and Plate XVI, Fig. 5. A series of transverse
sections of the proliferous stolon which is shown in longitudinal section
in the last figure, is given in Plate XX, Figs. 1, 2 and 3.

As the stolon lengthens the germinal mass also elongates, as is shown
at *n*, in Figs. 4 and 6 of Plate XLI, so that any transverse section of the
former, like those given in Plate XXI, cuts some portion, *m*, *n*, of the
latter. At first all the cells which enter into the composition of the germ-
inal mass are alike, and its structure is homogeneous, as shown in Plate
XX, Fig. 6, and Plate XLI, Fig. 7; but its peripheral cells soon become
arranged in an epithelium, Plate XX, Fig. 2, *m*; Plate XXI, *m*, and Plate

XVI, Fig. 5, *m*; which thus forms a follicle around a central core of cells, *n*. These central cells are the ovarian eggs. In young stolons, and at the root or proximal end of old ones, these eggs are small and crowded together, Plate XXXI, Fig. 4, but as we pass outward towards the tip of the stolon, Figs. 5, 6, 7, 8 and 9, they gradually grow larger, and the number cut by each transverse section grows less and less, until (Plate XXXIV, Fig. 4, *m*, and Plate XV, Fig. 2, *m*), they are pulled out into a series of single eggs.

The figures in Plates XXI and XXXI are from young stolons which are carried by embryos like those which are shown in Plate XLI, Figs. 3 and 5; but even in mature stolons, which have set free many generations of buds, there is an undifferentiated portion of the germinal mass at the root, as is shown in Plate XLI, Fig. 8, which is a little nearer the root of the stolon than the figures on Plate XXXIV.

On Plate XLI, Fig. 7, is a longitudinal section through the middle of the germinal mass of a very young embryo, before it has become differentiated into a central core of eggs and a peripheral follicle of epithelium. Figure 8 is a transverse section through the germinal mass of a fully grown stolon at its root, where the undifferentiated or embryonic germ cells are multiplying by karyokinesis, while Fig. 9 is from the same stolon a short distance from the root, where there is a continuous follicle, filled with ova, which latter have entirely lost the power of vegetative multiplication.

In both young stolons and old ones the undifferentiated germ cells multiply by indirect division, and one or two cells with nuclear figures may be found in each section, but as we pass towards the tip of the stolon and the central cells assume the characteristics of ova, Plate XXXI, Fig. 5, from a young stolon, and Plate XLI, Fig. 9, from a mature stolon, the egg cells cease to multiply, although they increase in size, both yolk and nucleus growing rapidly. The material for this growth is furnished by follicle cells, Plate XXXI, Figs. 5 and 6, which migrate from the peripheral layer, inwards among the egg cells, where they degenerate and break down.

In a stolon which is mature and ready to produce buds, the reproductive organ consists of a single row of fully developed ova, Plate XV, Fig. 2, *n*, surrounded by a follicular sheath which consists of an egg capsule of flattened cells, and an epithelium of thicker cells on the ventral or hæmal side of the eggs. See also Plate XXXIV, Figs. 2 and 4. The flattened cells give rise to the follicular capsule of the egg, Plate X, Fig.

1, *m*, and to the fertilizing duct, *x*, by which the egg is attached to the wall of the cloaca of the chain-salpa, as shown in Fig. 10. The thickened layer of epithelium gives rise to the testes.

As the constrictions which mark out the bodies of the chain-salpæ make their appearance in the walls of the stolon, the germinal mass also becomes divided up, Plate XV, Figs. 1 and 2, and Plate XXIII, Figs. 1, 2 and 5, into a series of segments, one for each chain-salpa. In Salpa pinnata, and in most species, each of these segments contains a single egg; but in those species which produce several embryos, such as Salpa cordiformis and Salpa hexagona. the number of eggs is the same as the number of embryos which is characteristic of the species. Thus the chain form of Salpa hexagona, Plate III, Fig. 1, normally gives birth to five embryos, and as shown in Plate XLV, Figs. 6 and 7, each segment of the genital string contains five eggs. Salpa democratica appears to be in an intermediate condition, for while it normally produces only one embryo, the segments of its genital string often contain, in addition to the single normal egg, one or two others which appear to be abortive, and which often exhibit indications of degeneration, although Salensky thinks there is reason to believe that a second embryo may be produced in this species after the first one has completed its development and has been set free.

As the body cavities of the chain-salpæ become separated from each other, the thickened epithelium under the egg becomes folded and pushed out to form two lateral pouches, Plate XV, Fig. 1, *m*, and Plate XXIII, Figs. 1 and 2, *m*, which are to give rise to the testes; and at a somewhat later stage a third fold or outgrowth appears on the middle line and ultimately becomes the fertilizing duct. This outgrowth, which is indicated in Plate XXV, Fig. 7, *H*, by the letter *m*, is derived from the epithelium of the follicle on the dorsal or neural side of the egg. In Plate XXV, Fig. 7, *G*, all three folds, the two paired testicular folds, and the unpaired median fold which represents the fertilizing duct, are shown, colored blue; and they are also shown in Plate XXVI, Fig. 1, *H* and *G*, and Figs. 2, *E* and *F*.

As the bodies of the chain-salpæ become developed and undergo changes of position, the reproductive organs also move away from their primitive positions, in a manner which will be readily understood from the figures. At the stage shown in Plate XXXIV, Fig. 4, the ovary is a continuous structure in a continuous sheath of follicle cells. At the stage shown in Plate XV, Figs. 1 and 2, and also in the reconstruction in Plate V, Fig. 1, the testicular folds, *m*, have appeared, and the follicle is con-

stricted into a series of segments with, in S. pinnata, and in most other species, one egg in each. This has gone a little further in the chain-salpæ shown in Plate XXVI, *A*, *B*, *C* and *D*, and in the reconstructions which are shown in Plate V, Figs. 2, 3 and 4, although the eggs still lie in a straight line in a plane which corresponds with the middle of the stolon. At a stage a little older, Plate XXV, Figs. 5, 6 and 7, *F*, *G* and *H*, and in the reconstruction in Plate VI, Figs. 1, 2, 3 and 4, the single row is broken up into two, and the eggs are carried alternately to the right and to the left, with the growth of the bodies of the salpæ, until they finally assume the positions shown in Plate XXXVIII, Figs. 95 and 99, *n*, and Plate VII, Fig. 4, *n*. The path which is taken by the egg in its migration, and its relations, and that of the testes, to the other organs of the body, will be fully described in the chapter on the process of budding. All that concerns us here is the attachment of the egg to the wall of the cloaca by means of the fertilizing duct, Plate XXXIX, Fig. 4, *x*, which ultimately becomes a tube, Plate X, Fig. 10, *x*, and Fig. 1, *x*, through which the spermatozoa reach the eggs. Most writers state that the egg is fastened to the wall of the pharynx, and it is difficult to decide, from the examination of adults alone, whether the point of attachment lies in the pharynx or in the cloaca, for there is nothing to mark the boundary between these structures, which are, however, more sharply separated in the young chain-salpa, where the duct is clearly seen to be attached to the wall of the cloaca.

The chain-salpæ of S. pinnata are set free in wheel-shaped or cylindrical clusters of eight or nine individuals each, Plate I, Fig. 2. At the time of birth each of them contains an unfertilized egg, essentially like the one from Salpa hexagona which is shown in Plate X, Fig. 1. The large nucleus with its network of chromatin threads and large nucleolus is surrounded by a granular yolk, which is enclosed by a capsule of follicle cells, which are now elongated, although they were so flat as to be scarcely visible at the stage shown in Plate XXXVI, Fig. 2. At one point the follicle is continuous with the fertilizing duct, *x*, which has, by most writers, been termed the oviduct, although there is no good ground for the use of this name, for no ova ever pass through it; and while it may possibly be homologous with the true oviduct of other tunicates, there is no evidence that this is the case, and I therefore prefer to use a name which at least has the merit of expressing its function, at the present day, as a channel for the spermatozoon.

Section 2.—*The Fœtal Membranes.*

Each egg gives rise to an embryo which becomes a solitary salpa, while the chain-salpæ are produced by budding from the solitary salpa.

The embryo is developed within the body of a chain-salpa, and its growth begins very soon after the chain-salpa is set free and while it is 'very small, and it keeps pace with the growth of the chain-salpa, so that a fully grown embryo is gigantic in comparison with the animal which carries it. Plate I, Fig. 3, is an individual of the chain-form of Salpa pinnata with its embryo, but in other species the embryo is relatively very much larger. Each chain-salpa usually contains only one embryo, as is shown in this figure, but in a few species there are several embryos in each.

Plate III, Fig. 2, is a side-view, and Fig. 3 a dorsal view of the chain-form of Salpa cordiformis, showing the embryos, *e m*, on the right side of the body, in the space between the fifth muscle and the sixth. Plate IV, Fig. 6, is a portion of a chain of the same species, showing, on the right side of the figure, the right sides of three salpæ, with five embryos in each, arranged in a row in the space between the fifth muscle and the sixth.

When there are several embryos they are in successive stages of development, as shown in Plate X, Fig. 10, which is from the chain-form of Salpa hexagona, shown in Plate III, Fig. 1, where the five embryos appear as a row of dots on the right side of the body, in the space between the last muscle and the next to the last.

The egg before it is fertilized, and the embryo during the early stages of its development, lies in one of the blood-channels of the chain-salpa: the space which is marked *y* in Plate X, Fig. 10, and in the other figures. The egg is suspended by the fertilizing duct, *x*, of Plate X, Fig. 10, which is fastened to the wall of the cloaca, *c*, into which it opens. The spermatozoa which are drawn into the pharynx of the chain-salpa with the sea water, are swept past this opening by the contractions of the muscles in swimming, and some of them enter it and, penetrating to the egg, fertilize it.

As the embryo grows it pushes in to the cavity of the cloaca, carrying its wall before it, as is shown in Plate XI, Fig. 3, where the letter *y* marks the blood-channel, while the *c* above the figure is in the cavity of the cloaca. The layer of epithelium which is marked *b'* is that part of the wall of the cloaca which is pushed in before the embryo, and becoming

closely wrapped around it, forms the covering which I shall call the *epithelial capsule* of the embryo. The way in which the growing embryo comes to project into the cloaca is also shown in Plate IX, Figs. 1 to 9, where the blood-space, y, is colored yellow, and the cavity, c, of the cloaca red.

The epithelial capsule covers the embryo during the early stages of development, and it is shown at b' in Plates XI, XII, XIII, XIV and XXII, and in cuts B, C and D. As it does not grow, it becomes distended by the growth of the embryo, and its cells grow more and more flat and farther and farther apart; and as the ectoderm is formed under it, it breaks up into separate cells which are thrown off as shown at b' in Plate XVI, Figs. 2 and 3, and in Plate XVII, Fig. 4, and at B' in Plate XLV, Fig. 3. Plate XVI, Fig. 6, is a part of Fig. 2 very highly magnified to show the formation of the ectoderm and the molting of epithelial capsule. In this figure, 15 is the body cavity of the embryo, a its ectoderm, and b' the detached cells of the epithelial capsule; 21 and 22 are the outer and inner folds of the embryo sac, which is to be described soon.

It will be seen that, at the stage shown in Plate XI, Fig. 3, that part of the embryo which is at the bottom is not covered by the epithelial capsule, but is directly exposed to the blood of the chain-salpa which circulates in the space y.

While this uncovered area subsequently becomes smaller, as compared with the growing embryo, it never becomes covered in completely, and within it the placenta is formed. This organ, which serves to nourish the growing embryo with food derived from the blood, is shown in Plate III, Fig. 4, pl, and in Plate XLI, Figs. 1, 2, 3 and 5, pl. It is also shown in longitudinal section in Plate XXXV at y''. It preserves its communication with the blood-channels of the chain-salpa until the embryo is born, and as this is nourished from the blood which passes into and out of the placenta, its function and its anatomical relations are strikingly like those of the mammalian placenta, although there is a very important difference which will be described soon.

The fold in the wall of the cloaca which covers the embryo and forms the epithelial capsule, soon extends down for some distance below the level of the embryo, as is shown in Plate XLV, Fig. 1, and forms the boundary of a spacious chamber, the cavity of the placenta, which opens through a constricted *neck* into the blood-channel of the chain-salpa.

The cells which compose this wall soon become elongated and thickened, as is shown in the figure at 23, while those which cover the

embryo and form the epithelial capsule are flat, as shown at *B'*. This difference becomes more and more marked and the transition more and more abrupt, until the lower thickened portion, cut *B, 23*, becomes sharply separated from the epithelial capsule *B'*, and forms what I shall call the *supporting ring* of the placenta. This is shown at various stages of development at *23*, in Plate XVIII, Plate XXXV and Plate XLV, and in cuts *B, C* and *D*.

One of its functions, and apparently the only one in most species, is to act as a framework for the placenta, and a support to hold the embryo in its position above the placenta; but it also has a nutritive function in at least one species, Salpa pinnata, and its cells ultimately degenerate and become converted into food for the embryo.

The rupture which sets the fully grown embryo free usually occurs around the neck of the placenta, so that the supporting ring is carried away with it and is gradually absorbed.

While the embryo projects into the cloaca of the chain-salpa as I have shown, it is not at first in direct contact with the water, for it is covered, in the first place, by the epithelial capsule, and in the second place by the *embryo sac*, which is now to be described. This structure is often called the *amnion*, as it bears a certain resemblance, in its anatomical relations, to the amnion of the higher vertebrates, although it is not formed, as it is in the vertebrates, from the tissues of the embryo, but from those of the chain-salpa. It first makes its appearance as a circular ridge or fold, Plate XLV, Fig. 2, *21* and *22*, in the wall of the cloaca, around the area where the embryo is attached by the neck of the placenta. In some species it seems to be absent; in others, as in Salpa hexagona, it is never any more developed than it is in the figure just referred to. More usually, however, it grows up around the embryo until this is completely shut in except for a small pore or unclosed space. It is shown at *21* and *22* in transverse sections of Salpa pinnata in Plate XVIII, Figs. 1–6, in longitudinal section in Fig. 8, and in surface view in Plate XLI, Fig. 1. The space between the embryo sac and the embryo is the *brood chamber*. In its origin this is part of the cavity of the cloaca, but it becomes completely shut off except for the pore, which is shown in Plate XVIII, Fig. 4. The wall of the embryo sac is double, and the space between its two folds is continuous with the blood-spaces of the chain-salpa.

It is plain from this description that a horizontal section, like the one in Plate XIII, Fig. 3, in the plane of the line marked XIII, 3 in cut, will

pass, first, through the outer fold, *21*, of the embryo sac ; second, through the space between the outer and inner fold, which is part of the body cavity of the chain-salpa, and is, like this, colored yellow; third, through the inner fold, *22*, of the embryo sac; fourth, through the brood pouch which is part of the cloaca of the chain-salpa and is colored red; fifth, through the epithelial capsule, *b'*, which is also colored red, and sixth, through the embryo.

The embryo sac is formed during the early stages of development, and it becomes complete while the embryo is very small; and as it does not increase in size, the rapid growth of the embryo soon causes it to distend, and the embryo soon pushes through the small opening, stretching this and forcing its way out into the cavity of the cloaca, as is shown in Plate XLI, Fig. 2, and in Plate XXXV.

As the epithelial capsule has in the meantime been cast off, the surface of the body of the embryo is now directly exposed to the water in the cloaca, and is fastened to its wall only around the neck of the placenta. At the stage which is shown in the last two figures the placenta is still inclosed in the embryo sac, although the embryo itself is free; but at the stages shown in Plate XLI, Figs. 3 and 5, the placenta also is uncovered, and the embryo sac is no longer recognizable, as its folds have been flattened out and obliterated by the growth of the embryo.

This brief outline of the history of the fœtal appendages is enough to make these structures intelligible in the figures of the various stages of development, and we are now in a position to trace the embryology of salpa from the egg, as our description need not be interrupted by references to these structures.

SECTION 3.— *The Migration of the Follicle.*

The salpa embryo consists of elements of two sorts: those derived from the fertilized egg, colored orange in the plates, and those derived from the follicle, which are, with a few exceptions, colored blue.

The egg, before fertilization, Plate X, Fig. 1, is inclosed in a capsule of follicle cells, *m*, which are, in ultimate origin, modified germ cells, as I shall show farther on. Each follicle cell is a cell which might have become an egg, although they are not now eggs. They are not fertilized, and while the part they play in the formation of the embryo is very remarkable and interesting, their function is purely nutritive, and they do not become converted into any of the tissues of the embryo. At first

the egg entirely fills the cavity of the follicle, as shown in Plate X, Fig. 1; but as soon as segmentation begins, after fertilization, Fig. 3, the cavity becomes divided into two portions, an empty one, *5*, and another which is occupied by the egg. Over the half occupied by the egg the follicle cells, *10*, retain their original character and their sharp boundaries, while those which form the wall of the empty half, *7*, lose their distinctness, and multiply rapidly by karyokinesis, so that this half soon becomes much thicker than the other. For a short time the blastomeres, Fig. 5, *9*, which are formed by the segmentation of the egg, are bounded on one side by the empty follicular cavity, *5*; but the follicle cells soon begin to migrate inwards in the zone which is marked *8* in Fig. 3, and soon completely cover up the blastomeres, as shown in Fig. 5, *7* and *8*, so that the follicular cavity is now bounded on all sides by follicle cells, and the blastomeres are imbedded in a mass of follicle cells. The follicle cells in the area which is marked *10* in Fig. 3, now begin to move inwards in radial lines and to push their way in among the blastomeres, and to force these apart, as is shown in Fig. 8, and this process of migration goes on until the follicular cavity is obliterated, as shown in Fig. 9. At the stage shown in Fig. 8, the embryo consists of the following structures: first, an outer or *somatic* layer of follicle cells, *7*; second, a *visceral* layer of follicle cells, *8*; third, an area, *10*, where these two layers are continuous with each other; fourth, the blastomeres, *9*; fifth, follicle cells between the scattered blastomeres, and sixth, the follicular cavity, *5*. In a stage a little older, Fig. 9, and Plate XI, Figs. 1 and 2, the cavity of the follicle becomes obliterated as already noted, and the follicle cells of the visceral layer begin to multiply very rapidly by direct division of the nuclei, although the somatic cells still multiply by karyokinesis, but much less actively.

Plate XLII, Fig. 1, is a part of the embryo, shown in Plate XI, Fig. 2, very highly magnified. Three blastomeres, *9*, and part of a fourth are shown imbedded in the mass of follicle cells of the visceral layer, *8*, which is not separated by any empty space from the somatic layer, *7*, of follicle cells. The blastomeres are so much larger than the follicle cells that they can be recognized without difficulty.

Each of them has a very large spherical nucleus, with a complicated network of very fine and delicate threads of chromatin. The nucleus is near the center of the blastomere, which is itself nearly spherical, very much more transparent than the surrounding follicle cells, with a well marked boundary, and filled with a number of bodies which stain much more deeply than the substance of the blastomere. The boundaries

between the visceral follicle cells are almost invisible, and their nuclei are elongated in radial lines, and are irregularly pear-shaped, with an aggregation of a substance, which stains very deeply, at the central end. These elongated nuclei are often arranged in pairs, the two members of the pair lying in the same radius, and many of them are in the act of dividing into an inner and an outer portion. In Fig. 2, which is from the somewhat older embryo which is shown in Plate XI, Fig. 3, *a* is a follicular nucleus which is about to divide, *b* is one in the act of division, and *c* is one which is separated into two daughter nuclei, of which the inner one has migrated inwards to a considerable distance towards the center of the embryo. In their migration some of these nuclei push their way in to the substance of the blastomeres, and in Fig. 1 one is shown in the act of penetrating its outer wall. Some of the bodies inside the blastomeres are sharply defined, and these agree with the follicle nuclei in size, in their color in stained specimens, and in the arrangement of their chromatin; and the study of sections at this and subsequent stages proves that the less sharply defined bodies are follicle nuclei in process of degeneration, and that the blastomeres are nourished by migratory nuclei from the visceral layer of follicle cells. The space between the blastomeres is also filled with these nuclei in all stages of degeneration and with the granules which have come from their disintegration.

The multiplication of the blastomeres goes on slowly, and while they gradually become smaller and more numerous, as shown in Figs. 3, 4, 5, 6 and 8, they are seldom found in the act of division. The material which is assimilated by the blastomeres from the migratory follicular nuclei, seems for some time to be converted into chromatin; for while the protoplasm of the blastomeres continues transparent, as shown in Figs. 2, 3 and 4, the chromatin of these nuclei increases in amount and forms a sharply defined reticulum with a large central nucleolus, and a number of smaller nucleoli around the periphery and in the meshes of the network. The nuclei of the follicular cells, on the other hand, become vesicular and transparent through repeated division, and their chromatin becomes more and more scanty, while they continue to divide so rapidly that they show a well-marked arrangement in pairs, as is shown in Fig. 3 and in the following figures. As the blastomeres continue to multiply they gradually become very granular, as is shown in Figs. 5, 6 and 8, and even after they have become nearly as small as the follicle cells, they are easily distinguishable by their large nucleoli and conspicuous network,

from the double, transparent, vesicular nuclei of the follicle cells, as is well shown in Fig. 8, where the blastomeres are marked *bl* and the follicle cells *7* and *8*.

Finally, the blastomeres begin to multiply actively by karyokinesis, as shown at *b* in Fig. 9, and to give rise to the germ layers; but as it is difficult to understand the peculiar relation between the follicle cells and the blastomeres, without some knowledge of the history of both structures, it will now be necessary to take up the history of the follicle before we study the history of the germ layers.

Stated in a word, the most remarkable peculiarity of the salpa embryo is this. It is blocked out in follicle cells which form layers and undergo foldings and other changes which result in an outline or model of all the general features in the organization of the embryo. While this process is going on the development of the blastomeres is retarded, so that they are carried into their final positions in the embryo while still in a very rudimentary condition.

Finally, when they have reached the places which they are to occupy, they undergo rapid multiplication and growth, and build up the tissues of the body directly, while the scaffolding of follicle cells is torn down and used up as food for the true embryonic cells.

No other animal presents us with an embryonic history quite like that of Salpa, although other Tunicates show something similar, but very much less pronounced. In another chapter I shall try to show how the life-history of Salpa has come about, but we must now confine ourselves to the facts.

An imaginary illustration may help to make the subject clear. Suppose that while carpenters are building a house out of wood, that brickmakers pile clay on the boards as they are carried past, and shape the lumps of clay into bricks as they find them scattered through the building where they have been carried with the boards. Now, as the house of wood approaches completion, imagine that bricklayers build a brick house over the wooden framework, not from the bottom upwards, but here and there wherever the bricks are to be found, and that, as fast as parts of the brick house are finished, the wooden one is torn down. To make the analogy complete, however, we must imagine that all the structure which is removed is assimilated by the bricks, and is thus turned into the substance of new bricks to carry on the construction.

SECTION 4.—*The Organs of the Embryo outlined in Follicle Cells.*

The structure of the young embryo and the shares which the two sorts of cells take will be understood from the plates, and especially from the horizontal sections in Plates XII, XIII, XIV, XVI and XVII, and from the diagrammatic reconstructions in cuts A, B, C and D.

The shape of the young embryo makes it difficult to control the position of sections in any plane except the horizontal; that is, the plane which is parallel to the bottom of the page in Plate XXXV. I have therefore paid especial attention to serial sections in this plane, and have figured a series from young embryos at successive stages of development, in Plates XI, XII, XIII, XIV, XVI and XVII. Longitudinal sections like Plate XXXV, and vertical transverse sections like those in Plates XVIII, XXII and XLV, are much more intelligible if they are perfectly symmetrical and exactly at right angles to the horizontal plane, but as the slightest deviation makes them hard to interpret, I have not been able to obtain, from young embryos, any which are exact enough to be useful for illustration. They have been valuable to me in the interpretation of the horizontal sections, but an attempt to describe them would complicate the description so much that I have not drawn them, but have, instead, reconstructed from them, and from the horizontal sections which are figured, the series of diagrams of vertical transverse sections which is shown in cuts A, B, C and D.

We left the follicle at the stage, Plate XI, Fig. 1, at which its cavity is entirely filled up by the visceral layer, *8*, which is in direct contact with the somatic layer, *7*, although the inner ends of the somatic cells are sharply defined. These two layers soon become separated again by a space, Fig. 3, *15*, which persists from this time as the permanent body cavity of the embryo. It is colored purple in all the figures, and is marked *15*. It is probably the original cavity of the follicle, opened a second time by the growth of surrounding parts, and at the stage of Fig. 3, and for a long time in the later history of the embryo, it is bounded on all sides by follicle cells. A diagram, constructed from a series of transverse sections of an embryo, like Fig. 3, is shown in cut A on page 29. In this, as in the plates, *y* is the body cavity of the chain-salpa, *B'* is the epithelial capsule, *15* is the body cavity of the embryo, *9* and *H'* are the blastomeres, and *7* and *8* the somatic and visceral layers of follicle cells.

On each side of the middle line the somatic layer is invaginated to form a pit which opens into the space between the embryo and the epithe-

lial capsule. This pit is the perithoracic tube or spiracular tube, and its external opening, which soon closes, is the spiracle. Before the two perithoracic tubes lose their external openings they elongate, and pushing across the body cavity into the substance of the visceral layer of follicle cells, they meet and unite on the middle line to form the cloaca.

Cut A.
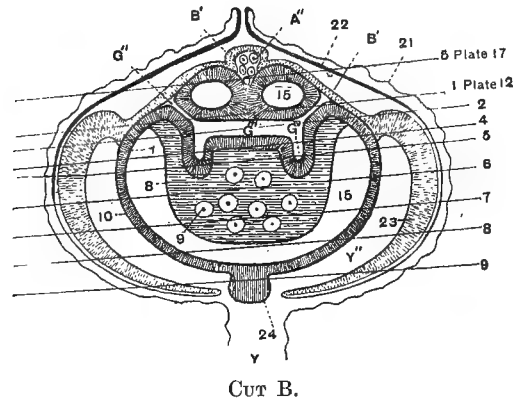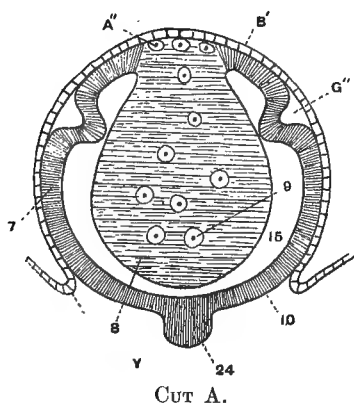
Cut B.

A reconstruction in a transverse vertical plane, from the horizontal section shown in Plate XVII, Fig. 5, and in Plate XII.

This stage in the history of the perithoracic tubes is shown in Plate XII, which is a series of horizontal sections of a young specimen of Salpa pinnata, and in cut B, which is a vertical transverse section constructed from the series of horizontal sections.

The reader who wishes to understand the structure of the embryo must compare these figures with each other. The double fold of the embryo sac, *21* and *22*, and the epithelial capsule, *b'* or *B'*, appear in all the sections, but as they have already been described, it only remains to point out that while these membranes are shown in the cut separated from the embryo by an empty space, as they are in the living embryo and in unshrunken specimens, they are represented in the horizontal sections as close to the surface of the embryo, as they are in specimens which have been imbedded in melted paraffine, which causes these delicate unsupported folds to shrink.

The plane of section 1, Plate XII, cuts the right spiracle *g''* close to its external opening, while it cuts the left one below its opening and just above the point where it communicates with the cloaca which is shown at *g'''* in section 2.

The epithelium which lines the cloaca and perithoracic tubes is derived from the somatic layer of follicle cells, but in the plates it is colored orange like the blastomeres, as I did not obtain proof of its folli-

cular origin until the plate was finished.   The orange color is used in the same way for the follicular epithelium of the cloaca and perithoracic tubes in Plate XXII.

Below the level of the cloaca the perithoracic tubes run downwards, one on each side of the middle line as shown at g' in Fig. 3 and at g^v in the cut, but they end blindly at this stage, and in Fig. 4 the left is cut at its end below the cavity, while the right, g, is cut close to the bottom of the cavity.   The next section, Fig. 6, does not cut any part of the perithoracic tubes, although their positions are outlined on each side of the middle line in visceral follicle cells with scattered blastomeres.   The next stage in the development of the perithoracic system is shown in Plate XIII and in cut C.

CUT C.
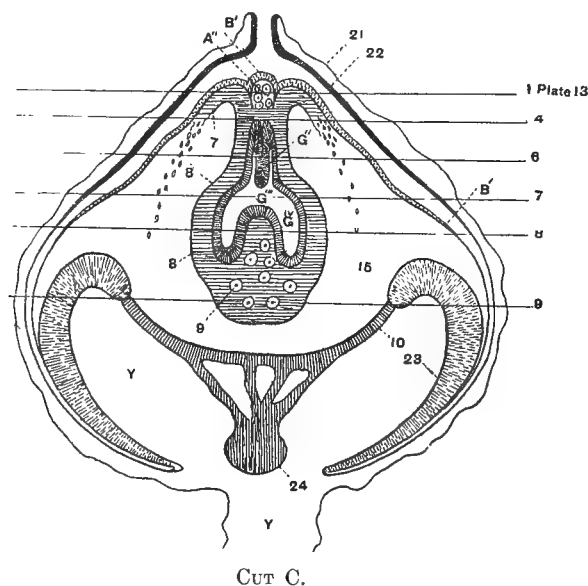
A reconstruction in a vertical transverse plane from the horizontal
sections shown in Plate XIII.

The cloaca, Fig. 7, g''', and the two prolongations of the tubes downwards, Fig. 8, g^{IV}, are about as before, except that these latter are a little longer.   The spiracular tubes, however, have undergone a great change, as they have lost their external openings and have moved towards the middle line, where they lie side by side at g'', in Fig. 6, above the cloaca, imbedded in the visceral follicle cells.

In Plate XIV and in cut D, the pharynx, c, is shown, as well as the two gill-slits, g^{IV}, which have been formed out of the descending portions of the perithoracic tubes, Fig. 6, g^{IV}, which now open above into the cloaca, Fig. 5, g''', and below into the pharynx, Figs. 8, c and 9, c.   In Figs. 3 and 4, the two spiracular tubes g'' are shown side by side, above the cloaca,

imbedded in visceral follicle cells. Plate XXII shows a series of trans-
verse sections at the same stage, but as these are not perfectly vertical
but in planes which make an acute angle in front with the horizontal
axis, they are less easily intelligible than the cut. Fig. 2 passes through
the pharynx, c; Fig. 3, through the two gill-tubes, g'', and Fig. 4, through
the cloaca, g''', and the two spiracular tubes, g'', g''. The details of the
formation of the pharynx and gill-tubes and gill, o, are shown in Plate
XLII, Figs. 6, 7 and 8. The cavity of the pharynx, Fig. 8, c, and Plate
XIV, Figs. 8 and 9, c, and Plate XXII, Fig. 2, c, is hollowed out in the
mass of visceral follicle cells, below the cloaca, by the degeneration of
the follicle cells. These become amœboid and are set free in the cavity,
where they persist for some time. They are easily recognizable by the
transparency of their nuclei, and by the fact that these are usually in pairs.
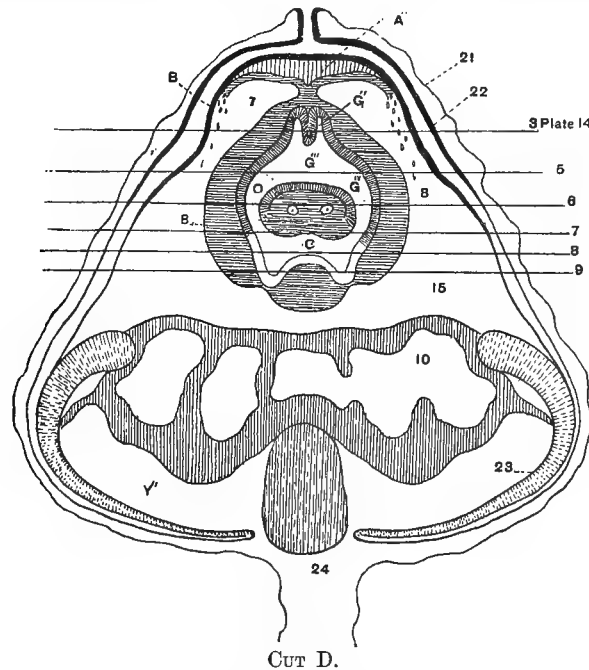
Cut D.

A reconstruction in a vertical transverse plane from the horizontal
section shown in Plate XIV.

The communication between the perithoracic tube g'' and the
pharynx is formed in essentially the same way. The somatic follicle
cells, 7, of the perithoracic tube, Fig. 6, Plate XLII, and Fig. 8, and
the visceral follicle cells between it and the pharynx, Fig. 8, 8, become
amœboid, and wander out into the cavity which is thus formed. The
rod-like mass of cells, Fig. 8, o, which is left between the cloaca g, above,
and the pharynx c, below, and the gill-slits g'', on the sides, is the so-called
gill. It consists of a mass of visceral follicle cells, 8, which contains

scattered blastomeres, *bl*, and is covered above and at the sides by a layer of somatic follicle cells, 7.

The degeneration of the follicular lining of the cloaca begins before the gill-slits are formed. Fig. 7 of Plate XLII is part of Plate XIII, Fig. 8, showing part of a perithoracic tube at the stage when it ends blindly. The visceral follicle cells are shown on the left; then the follicular epithelium of somatic cells, and then three of these cells, with double nuclei, which have become amœboid, and have wandered into the lumen of the tube. Fig. 6 is a section through the axis of the gill-slit, from a specimen at the same stage as Plate XXII, showing the follicle cells breaking apart to form the channel of communication with the pharynx.

As the embryo grows the pharynx increases in size, at first, very much faster than the cloaca, and at the stage shown in Plates XVI and XVII, the pharynx, Fig. 2, *c*, is very capacious, while the cloaca, XVI, Figs. 1 and 2, g'' and g^{iv}, is very small. The relative sizes of the two structures are also shown at a somewhat later stage in Plate XVIII, Figs. 4 and 8, *c* and g''', and also in the surface view in Plate XLI, Fig. 2, *c* and g'''. The two spiracular tubes seem to fuse into one by the disintegration of the partition, as shown in Plate XVI, Fig. 1, g''; and their chamber becomes part of the cloaca. All the somatic follicle cells of the cloaca ultimately fall into its cavity and degenerate, although this process is not completed until the other organs of the body are well advanced in their development, and they are shown in Figs. 1, 2 and 3 of Plate XVI, and at *x* in Fig. 9 of Plate XLII.

SECTION 5.—*The Blastodermic Tissues of the Embryo.*

We have now to consider the way in which the blastodermic epithelium of the pharynx and cloaca replaces this temporary scaffolding of follicle cells.

While the changes which we have described are taking place, the blastomeres gradually become smaller and more numerous, as shown in Plate XLII, Figs. 1, 2, 3, 4, 5, and those which are to give rise to the epithelium of the pharynx and cloaca become distributed through the mass of visceral follicle cells under the region of the perithoracic tubes and cloaca, as shown in Plate XII, Figs. 6 and 7, *9*, and in Plate XIII, Fig. 9. As the cavity of the pharynx is formed they become arranged between the visceral follicle cells, Plate XLII, Fig. 8, *8*, and the degenerating somatic cells, 7, as is shown at *bl* in Fig. 8 and in Fig. 5. In this way the

epithelial lining of the pharynx is gradually completed and extended until at last it becomes continuous, as shown in Fig. 9, in which b is the cavity of the pharynx and *15* the body cavity. Even after this epithelium is well defined, it is here and there interrupted by a follicle cell, with its two transparent nuclei, as is shown at *8* in Fig. 10. This figure is part of Fig. 2 of Plate XVII.

Other blastomeres migrate upwards along the gill-slits, under the somatic cells, as shown in Fig. 6 of Plate XLII, which is from an embryo at the stage of Plate XXII. These cells multiply, and finally build up a continuous epithelium in the gill-slits, as shown in Plate XLII, Fig. 9, which is part of the section shown in Plate XVI, Fig. 3. In Fig. 9, g^{iv} is the cavity of the gill-slit, x is a group of degenerating somatic follicle cells, b' is the blastodermic epithelium, *15* is the body cavity, *8* the visceral follicle cells, A mesoderm cells, a the ectoderm, and b' the cells of the degenerating epithelial capsule. The œsophagus, stomach and intestine are formed from a diverticulum from the posterior wall of the pharynx, a little to the right of the middle line, as shown at a very early stage at q in Plate XVII, Fig. 2, and more magnified in Plate XLII, Fig. 10, q. The opening of this diverticulum becomes the œsophagus, shown at q in Plate XVIII, Figs. 6, q and 8, q, and it elongates to form the stomach and intestine, but as it soon becomes twisted it cannot be described in detail without a greater number of figures than the subject seems to merit, as the only point which seems noteworthy is that the digestive tract is a secondary outgrowth from the pharynx. As the blastodermic epithelium of the gill is formed, the intestine extends forwards into it, as is shown in Plate XLI, Figs. 3 and 5, and at p in Plate XXXV. The anus is not formed until the embryo is well advanced, when it breaks through into the cloaca at the point marked p'' in Plate XXXV, on the middle line at the anterior end of the cloaca. Plate XIX, Fig. 1, is a horizontal section, reversed in the drawing, through the gill of an embryo a little older than Plate XXXV, showing the intestine p in the gill o, and the opening of the œsophagus q, on the right side at the base of the gill.

At a very early stage, Plate XVII, Figs. 1 and 2, a stomodæal involution of the ectoderm z grows inwards to meet a corresponding outgrowth from the pharynx, although the aperture of the mouth is not formed until the embryo is well advanced. In Plate XXXV the stomodæum z is shown with its inner end covered up by the unbroken epithelium of the pharynx. Plate XLV, Fig. 8, is a vertical section through the mouth at the time of its appearance, from an embryo like Plate XLI, Fig. 5.

In Plate XLV, Fig. 8, v is the cellulose mantle which is not yet perforated, and c is the cavity of the pharynx. The ectoderm and endoderm are united at the edge of the lower lip, but they are as yet separate in the upper lip, which is rounded and thick and protuberant, with three transverse muscles, w, and at a later stage the thin lower lip is tucked inwards under the rounded upper lip, to form the oral valve of the branchial sac.

The gill of salpa, Plate XLII, Fig. 8, o; Plate XVIII, Fig. 4 and Fig. 8, o, Plate XXXV, o, and Plate XLV, Fig. 4, o, is simply the space bounded by the pharynx below, the gill-slits at the sides and the cloaca above. It is at first a solid mass of follicle cells with blastomeres which give rise to its lower or endodermal epithelium, but its sides are only slowly covered with blastodermic epithelium, and its upper surface consists of follicle cells in embryos which have acquired most of their organs. The follicle cells in its cavity degenerate as the intestine extends into the gill.

The cloaca is at first lined throughout by somatic follicle cells. Some of these begin to migrate into its cavity very early, and in some specimens the whole chamber is so choked up with them that it is difficult to trace it. The blastodermic epithelium gradually extends over its whole surface, and the follicle cells degenerate and disappear. Its cavity then enlarges very rapidly, as will be seen by comparing Fig. 8 of Plate XVIII with Plate XXXV, or by comparing the embryos on Plate XLI. After its epithelial lining is complete, the ectoderm, Plate XVII, Fig. 6, a, bends downwards around a circular area to meet it, in such a way that a lump of visceral follicle cells is shut in under an arched cover of ectoderm. These cells then become vacuolated and finally disappear, as does also the cap of ectoderm. The ectoderm around the edge of the circle now bends inwards upon itself as shown in Fig. 7, and becomes continuous with the cloacal epithelium, and all of this inside the circular line of adhesion degenerates as shown in the figure, and finally disappears, to form the cloacal aperture. Figure 6 of Plate XVII is from the embryo shown in Plate XXXV, and Fig. 7 from one like Plate XLI, Fig. 3.

It will be seen from this account that the cloacal aperture is a new formation, and that it is not the two spiracles united into a single aperture, although I believe that the history of salpa is quite reconcilable with the view that it is phylogenetically a pair of spiracles. The spiracles which are formed in the somatic layer of the follicle lose their external openings, as we have seen, and the spiracular tubes move towards the middle line and unite at the spot, above the cloaca, where the aperture is

afterwards formed. They are not blastodermic but follicular, and they therefore do not repeat the ancestral history in all details, but their changes of position are quite intelligible on the assumption that they are a record which has been preserved from a time when the spiracles themselves moved up to the middle line of the back and fused to form the cloacal aperture.

The changes which take place in the position of the aperture during the growth of the embryo are most interesting. The first trace of it, Plate XLI, Fig. 2, g^v, is at the upper end of the long vertical axis of the embryo, and the space between it and the ganglion, s, is about equal to the space between the ganglion and the mouth, z; and the axis of the mouth and that of the cloacal aperture make an angle of about 90°.

As the embryo grows, Figs. 3 and 5, the mouth and the ganglion preserve essentially their original relations to each other, but the space between the ganglion and the cloacal aperture gradually increases until, at last, mouth and cloacal aperture come, in the adult Salpa pinnata, to lie in the same axis at opposite ends of the body, as is shown in Plate I, Fig. 1.

In the fixed ascidians the mouth and the cloacal aperture are close together, with the ganglion between them, and in this, as well as in other respects, the young salpa embryo is much more like a fixed ascidian than the adult, and I think we must see, in the primary position of the cloacal aperture, evidence that the salpæ are descended from fixed ascidians, or, at least, from ancestors very similar to the ascidians in structure and habits.

We have now traced the broad outlines of the history of the digestive organs, and of the perithoracic tubes and their derivatives, and we will pass to other systems of organs.

Returning to the stage shown in Plate X, Fig. 8, we have seen that the outer wall consists at this time of a somatic layer of follicle cells, 7, which is continuous, over the area 10, with the central mass of follicle cells, 8, and blastomeres, 9. The area, 10, where the two layers are continuous, marks what is to be the middle line of the dorsal surface of the embryo, and some of the blastomeres soon move outwards along this line until they pass entirely out of the follicle and lie directly under the epithelial capsule. These cells are the ectodermal blastomeres, and they are shown at a'' in cuts A and B and in Plate XXII, and at 9' in Plate XVII, Fig. 5, and in Plate XII, Figs. 1 and 2. The epithelial capsule, which at first passes over them without interruption, as shown in cut A, soon becomes

folded down on each side of them, so as to form a median dorsal ridge, shown in cut B and in Plate XVII, Fig. 5. At first, as is shown in cut A, these ectodermal blastomeres are not abruptly separated from those at deeper levels, but as the perithoracic tubes and their derivatives are formed and grow inwards, they separate the ectodermal blastomeres, in the middle region of the body, from those of the visceral mass, as is shown in cuts B and C.

The series of sections on Plate XII shows, however, that there is no such interruption before or behind the cloaca. At the posterior end of the body, the end which is at the top in the figures, a continuous series of blastomeres may be traced through all the sections, from *9'* in Plate XVII, Fig. 5, through *9'* in Plate XII, Figs. 1 and 2; and *18* in Figs. 4 and 5 to *18* in Fig. 6. At the anterior end a similar series of blastomeres may be traced from *9* in Plate XVII, Fig. 5, through *9'* in Plate XII, Fig. 1; *9* in Fig. 2, and *s* in Figs. 4, 5 and 6, to Fig. 7.

The mass of visceral follicle cells and blastomeres which makes up the embryonic region is at first nearly spherical, as shown in the diagram in Plate XII, Fig. 10.

As the embryo grows and the body cavity becomes more capacious, the visceral mass becomes folded into two vertical plates, intersecting at right angles in such a way that a horizontal section shows it as a cross, Plate XII, Fig. 5, with its long arm in the middle plane of the embryo, and crossed near its anterior end by the short arm. In Fig. 1 the ends of the short arm are continuous around the spiracular openings of the perithoracic tubes, with the somatic layer, as they are also in Fig. 2, where it contains the cloaca, which, with its lining of somatic cells, runs across the middle line and perforates the long arm of the cross. In Fig. 4, the enlarged rounded ends of the short arm contain the blind ends of the perithoracic tubes, as also in Fig. 5, while in Fig. 6 and in Fig. 7 the short arm contains the blastomeres which are to form the pharynx or branchial sac, into which, at a later stage, the perithoracic tubes open through a single gill-slit on each side. The long arm of the cross is formed by what is shown by the series of sections to be a thin vertical plate of visceral follicle cells and blastomeres, hanging down into the body cavity from an area on the middle line of the dorsal surface where it is suspended from the layer of somatic follicle cells. In the middle region of the body this vertical plate is interrupted by the perithoracic organs, and it is perforated by the cloaca, but both below and above the cloaca it is a continuous plate.

At the level of Figs. 5 and 6 which are in the planes indicated by the lines *5* and *6* in cut B, the whole cross lies free in the body cavity. At the level of Figs. 4 and 2 the ends of the short arm become continuous with the somatic layer, and the long arm is interrupted by the cloaca.

In Fig. 2 the posterior end of the long arm becomes continuous with the somatic layer, as does its anterior end also in Fig. 1, where the long arm is continuous above the cloaca, as it is below it in Fig. 4.

Most of the organs of the body except the perithoracic system are outlined in the visceral follicle cells of the median plate, and the blastomeres are grouped with reference to this outline.

At the level of Fig. 4 there are three well marked enlargements of the median plate, *s, 19* and *18.* One of these, *s,* lies anterior to the transverse plate, and is the rudiment of the ganglion. In Fig. 2 it is about as in Fig. 4, but in Fig. 1 its follicle cells become continuous with those of the somatic layer, and in Plate XVII, Fig. 5, its blastomeres, *s,* become continuous with the extra-follicular blastomeres of the ectodermal ridge which is shown at *A"* in cut B. The enlargement of the median plate at its posterior end, *18,* is the rudimentary nervous system of the caudal region, and in Figs. 2, 1, and XVII, Fig. 5, its blastomeres can be followed up into the posterior end of the ectodermal ridge, just as those of the ganglionic rudiment can be followed up into its anterior end. The third thickening, which is marked *19* in Plate XII, Fig. 2, is the rudiment of the notochord. At the level of Fig. 2 it contains no blastomeres ; at the level of Fig. 3 it contains two blastomeres, but there are none in Fig. 4, while they are numerous at lower levels, as is shown in Fig. 5 and also in Fig. 6, where the notochord is erroneously marked *18* instead of *19.*

At the level of Figs. 6 and 7 the median plate, with its blastomeres, swells out into a pair of lateral lobes which lie under the blind ends of the perithoracic tubes, and give rise to the pharynx, as already described.

All these structures are shown, in essentially the same relations, in the older embryo in Plate XIII, except that the anterior end of the body is at the top of the figures, and the caudal nervous system is marked *30* instead of *18,* in both Plate XIII and Plate XIV.

The most important changes in the visceral mass at this stage concern the perithoracic system, and have already been described.

The follicular rudiment of the caudal nervous system is also beginning to break down and disappear. The degeneration begins at the upper end, where it is continuous with the ectodermal ridge, and at this stage, as shown in Plate XIII, Figs. 6 and 7, *30,* it is now represented only

by scattered cells, although it is still sharply defined at a lower level, as is shown in Figs. 8 and 9, *30*. The next older embryo, Plate XIV, shows it in about the same condition, degenerated and with no distinct boundary in Figs. 4, 5 and 6, *30*, but sharply defined in Figs. 7, 8 and 9, *30*. I do not know what becomes of its blastomeres, but as they gradually become unrecognizable, I see no reason for supposing that they persist, and they probably degenerate and disappear.

The structure which is marked *19* in Plates XII, XIII and XIV becomes the larval organ which is generally known in salpa as the eleoblast. Its successive stages of development in Salpa pinnata are shown at *k* in Plates XVI, XVII, XXXV, XIX, and in Plate XLI, Figs. 2, 3 and 5, *k*. As these plates show, it grows with the growth of the embryo, and is in the older larvæ a prominent organ of considerable size.

Salensky suggests that it may be a rudimentary tail, and while he does not describe its structure minutely nor present much proof, his view is unquestionably the true one. In Salpa pinnata degenerative changes begin in it very early, at the stage of Plate XVI, and go on as it grows, so that its internal structure is always vague and indefinite, but it is very much less rudimentary in the embryos of other species, especially those of the *cordiformis* group, and sections of it, in advanced embryos of these species, show that it is unquestionably the embryonic and degenerated representative of the locomotor tail of the tadpole larvæ of other ascidians. In Plate III, Fig. 4, I have drawn an advanced embryo of Salpa hexagona, and in Plate VIII, Fig. 3, I have copied for comparison one of Uljanin's figures of the tailed larva of Doliolum, showing the chorda *ch*, and the caudal vesicle *k*, which is formed by its degeneration.

Comparison of these figures shows clearly that the position and the anatomical relations of the eleoblast, *k*, of Plate III, Fig. 4, are identical with those of the tail-vesicle *k* of the Doliolum larva. Fig. 1 of Plate XLIV is a transverse section through the eleoblast of the embryo of Salpa hexagona which is shown in Plate III, Fig. 4. This figure shows that it consists, first, of the outer sheath or cellulose mantle, *v*; secondly, of a layer of very thin flat ectoderm cells, *a*; third, of a circular tract of the body cavity, *15*, filled with blood corpuscles, *bl*, and migratory follicle cells, *29*; and fourth, of a great mass of wedge-shaped or subconical chorda cells, *k*, radially arranged, with all their protoplasm, and their nuclei, at their outer ends, while the empty bodies of the cells converge towards the center. These conical cells are wedge-shaped in section, but

as their position is not perfectly radial, but inclined to the axis of the chorda, a section does not lay open the whole length of any one cell from periphery to center. The axis of the chorda is occupied by a mass of granular protoplasm with scattered nuclei.

In an ordinary ascidian the tail degenerates before the definitive structure of the adult is acquired, but in Doliolum, as Uljanin's figures show, it is retained for a time after the little animal has ceased to be, in other respects, a larva, and has completely acquired all the characteristic structures of the adult Doliolum.

In this respect Salpa agrees with Doliolum, for the young Salpa hexagona, in Plate III, Fig. 4, is not a larva, but a perfectly formed young salpa, although it still retains its notochord *k* and its placenta *pl.*

Other species of salpa retain the rudimentary tail still longer, and there is a trace of it in the adult Salpa cordiformis. Plate IV, Fig. 3, is a young specimen of the solitary form of this species, some time after birth, showing the tail as a distinctly marked outgrowth of the cellulose mantle, with a cavity which is part of the body cavity. There is no trace of the chorda, although comparison with embryos of this species and of Salpa hexagona shows that this process is the same as the tail of Fig. 4 of Plate III. It grows smaller compared with the body, as the animal grows up, but it is easily seen even in the adult Salpa cordiformis, Plate IV, Fig. 5.

In Salpa pinnata the tail reaches considerable size, as is shown in Plate XLI, Fig. 5, but degenerative changes occur in it so early that its internal structure is vague and indefinite. It consists, as is shown at *k* in Plate XXXV and in Fig. 9 of Plate XIX, of an outer wall of flattened ectoderm cells, *a*, which is covered by a thin layer of cellulose, and forms the wall of a chamber which has groups of blood corpuscles around its periphery, while its central portion is occupied by a vacuolated mass of irregular cells in process of degeneration, and this species, studied alone, would give little evidence as to its nature, although other species show clearly that it is a tail, and that its central mass is a somewhat degenerated notochord. It now remains to trace its origin from the body which is marked *30* in Plates XIII and XIV, and *19* in Plate XII. Plates XVI and XVII show it, in a transitional stage, at *k*. In this embryo all traces of the caudal nerve have disappeared, and the notochord is represented by a sharply bounded mass of cells filled with large vacuoles. I was not able to trace the history of the blastomeres, but as the circular vacuoles make their appearance at about the time when the round blastomeres

disappear, they are possibly the spaces which they occupied. If this is true, the rudiment of the true blastodermic chorda degenerates in Salpa pinnata before its follicular case, but both structures soon break up, and are used as food.

We have traced the migration of the ectodermal blastomeres from the interior of the visceral mass to their extra-follicular position in the ectodermal ridge, Plate XVII, Fig. 5, *9*, and cuts B and C, *a''*. In this position they are covered up by the epithelial capsule, but are on the outside of the embryo. At a stage a little older than Plate XIV and Plate XXII, they begin to multiply and to spread out over the embryo on both sides of the middle line to form the ectoderm. Plate XLII, Fig. 11, is a transverse section of the ectodermal area of an embryo a little older than Plate XIV. *21* is the outer fold of the embryo sac, *22* its inner folds, and *b'* is the epithelial capsule; *a* is the ectoderm spreading out at the sides between the epithelial capsule and the somatic layer, *8*, of follicle cells. At this early stage in the development of the ectoderm its cells and nuclei are so much larger than those of the follicle that they can be distinguished clearly, and the nuclei of the blastoderm cells are rich in chromatin and have a well marked reticulum with nucleoli and large granules, and are in this quite different from the vesicular nuclei of the follicle cells. The ectoderm has a growing edge, like that of a meroblastic embryo, and it gradually spreads on all sides, and pushing under the separated cells of the epithelial capsule, forces them off, and thus finally becomes the outer covering of the embryo. The embryo, which is shown in Plates XVI and XVII, is almost covered by the ectoderm, and Figs. 6 and 7 of this plate and 9 of Plate XLII are parts of this embryo, more highly magnified to show the details of its structure. In all these figures *a* is the ectoderm, and *b'* the epithelial capsule. The cellulose mantle makes its appearance as soon as the ectoderm is fully formed, as a transparent layer, which is shown at *v* in Plate XVII, Fig. 6, and in Plate XLV, Figs. 3, 4 and 6.

A few small cells are sometimes included in it, as in Plate XVII, Figs. 5 and 6, *v*, but the cells of the epithelial capsule are on the outside of it, as is shown at *v* in Plate XLV, Fig. 3.

We have seen that the blastomeres of the ganglionic rudiment, *s*, are at first continuous, above with the extra-follicular blastomeres of the ectodermal ridge, as shown in Fig. 5 of Plate XVI, where *s* marks the ganglionic blastomeres. At a lower level, Plate XII, Fig. 1, *s*, they are imbedded in follicle cells at the point on the middle line of the body

where the visceral follicle cells are continuous with those of the somatic layer. In Fig. 2, *s*, they are shut into the visceral layer, and the ganglionic rudiment lies in the body cavity, *15*. At a still lower level, Figs. 6 and 7, the blastomeres pass without any line of demarcation into those of the pharynx.

In an embryo a little older, Plate XIII, we find essentially the same condition. In Fig. 3, *s*, the ganglion cells are extra-follicular; in Figs. 4 and 5, *s*, they gradually pass into the visceral layer of the follicle; in Figs. 6, 7 and 8, the ganglionic rudiment is within the body cavity, and in Fig. 9 it becomes continuous with the rudiment of the pharynx. In Plate XIV, Fig. 4, *s*, it begins to be shut in dorsally and separated from the somatic layer, while a cavity appears within it, as shown in Figs. 5 and 6, and in Plate XXII, Fig. 2, *s*. The cavity of the pharynx, *c*, is formed at the same time, and at first consists of a broad chamber, Plate XIV, Figs. 8 and 9, and a much narrower anterior portion, which is shown in Fig. 8 and, at an older stage, in Plate XVII, Fig. 1. This narrow portion, which is the stomodæal portion of the pharynx, soon loses its distinctness, as the whole pharynx widens out directly up to the mouth, as shown in Plate XIX, Figs. 4 and 5, but before this takes place the stomodæal diverticulum lies directly under the ganglion, as will be seen by comparing Figs. 6, 7 and 8 in Plate XIV, although the cavities of the two structures are at first separate, as shown in the intermediate section, Fig. 7.

The follicle cells which separate them soon disappear, and the cavity of the ganglion opens into the cavity of the stomodæal diverticulum of the branchial sac. This is the case at the stage which is shown in Plates XVI and XVII, where the cavity of the ganglion is shown at *s* in Figs. 2 and 3 of Plate XVI, while the stomodæal diverticulum is shown in the same place in Fig. 1 of Plate XVII. The study of the intermediate sections, which are not figured, shows that there is now no partition between the two, and the relations between the two structures are as shown in a longitudinal section of an embryo a little older, in Plate XVIII, Fig. 8, *s* and *z*. The opening of the ganglion into the pharynx is also shown in Plate XVIII, Fig. 2, where *s* is the cavity of the ganglion and *c* the cavity of the pharynx. Plate XXXV, *s*, shows the ganglion at a still older stage, and its opening into the pharynx at *t*, in longitudinal section.

The pericardial rudiment first makes its appearance in the longitudinal plate of the visceral mass, at *f*, in Plate XIV, Figs. 8 and 9, between

the notochord and the pharynx. It quickly becomes a large hollow vesicle, Plate XVIII, Figs. 1, 2, 3 and 4, *f*, which runs vertically behind the pharynx. On the side next the pharynx the heart, *e*, is formed as a vertical groove or fold in the wall of the pericardium, which ultimately becomes converted into a tube by the union of its edges, as shown at *c* in Plate XIX; the union first taking place in the middle, and extending towards the ends, which are permanently open and communicate with the space of the body cavity in which the blood circulates. As the heart becomes shut in, the follicle cells which lie between it and the wall of the pharynx, as shown in Plate XVII, become folded into it, as shown at *e* in Plate XIX, Figs. 8 and 9, and they then become vacuolated, and ultimately disappear. At the stage shown in Plate XIX the heart has essentially its adult form, and the series of sections illustrates its position and anatomical structure with sufficient clearness. Figure 1 is near the top of the heart, and shows its opening into the body cavity, which is shown in Fig. 6 more enlarged and filled with blood corpuscles, which are formed in the body cavity and are drawn into the heart by its pulsations.

Fig. 2, which is shown more magnified in Fig. 8, and Fig. 3, which is shown more magnified in Fig. 9, show the structure of the middle region of the heart, while Fig. 5 shows its inferior opening into the body cavity. The heart changes its position with the growth of the embryo. It is at first behind the pharynx, as shown in Plate XLI, Fig. 2, *f*, but as the pharynx elongates it pushes the heart down and grows over it, as shown in Plate III, Fig. 4, *e* and *f*, where the heart is in its adult position under the posterior end of the pharynx.

I have not been able to trace the origin of the mesoderm, and have first found it at the stage shown in Plate XLII, Fig. 8. At the lower part of this figure a number of small cells, with deeply stained nuclei, much smaller than those of the follicle cells, are shown in the body cavity, *y*, outside the visceral follicle cells, and other similar cells, *mes*, are shown arranged in an epithelium on the surface of the visceral cells, *8*. These are mesoderm cells, some of which are shown more magnified at *A* in Fig. 9, and in Plate XLII, Fig. 7. Their position seems to indicate that they are derived from the endodermal blastomeres of the pharynx.

SECTION 6.—*The Degeneration of the Follicle.*

We must now turn back from the history of the embryonic cells and their derivatives and trace the fate of the follicle cells. At a very early

period in the history of the embryo some of the visceral follicular nuclei wander in among the blastomeres, or push into their substance, as is shown in Plate XLII, Fig. 1, and there degenerate, as has already been described.

The stage which is shown in Plate XII and in cut B is the starting point for a description of the fate of the others, since the follicle is at this time most fully developed, and consists, as already described, of a somatic layer, 7, which forms the outer wall of the embryo; a visceral mass, 8, and the somatic lining of the perithoracic structures, g, g', g'', g''' and g^{IV}.

The somatic layer of the follicle is divisible into two parts, the upper portion, cut A, 7, which is covered by the epithelial capsule, B', and the lower part, 10, which is exposed to the body cavity, y, of the chain-salpa. These two regions are so different in their history that they must be treated separately. The lower portion, 10, forms the roof of the placenta, cuts A and B, 10, and, as is shown in cuts C and D, it soon loses its continuity with the upper portion, and establishes a union with the upper edge of the supporting ring, 23, which, as I have shown, itself loses its continuity with the epithelial capsule, B', and bends inwards, as shown in cut C, to unite with the follicular roof of the placenta. In Plate XII, the cavity of the placenta, colored yellow, is shown at y'' in Figs. 2, 4, 5, 6, 7, 8 and 9, and the supporting ring, 23, which is colored red, is shown in the same figures, where the part of the somatic layer of the follicle which is to enter into the formation of the roof of the placenta is marked 10. The relations between these various structures are so well shown in the figures and in the reconstructed cross section in cut B that description seems unnecessary, but we must note that the upper edge of the placenta is not a flat circle, but that it reaches much farther up on the sides of the embryo than it does on the middle line, and that the line which separates the placental portion of the somatic layer from the embryonic portion follows the same course, as does also the zone of transition from the epithelial capsule to the supporting ring. Comparison of cuts A, B, C and D also shows that as the embryo grows, the roof of the placenta becomes flat, and that the embryo itself pushes upwards from the surface which is thus formed.

The fate of the follicular cells of the roof of the placenta will be described in the account of the placenta, and we have now to consider the fate of those follicle cells which are more intimately bound up in the structure of the embryo.

At the stage shown in Plate XII, the somatic follicle on and near the middle line, just above the placenta, at 7, in Figs. 4 and 5, begins to lose its cell outlines and to become thickened and indefinite, and, as is shown at 7 in Plate XIII, Fig. 9, it very soon breaks up into its constituent cells, which separate from each other and become amœboid, although most of them remain in the places which they occupied at an earlier stage. This separation of the cells is accompanied by the formation of a transparent gelatinous substance between them, so that they become converted into a cartilage-like substance which lies around the circumference of the body cavity of the embryo just above the placenta, and most abundant near the middle axis, as is shown at *fc* in Plate XVIII, Figs. 1 and 8, and also in Plate XXXV. The disintegration of the somatic layer gradually extends upwards, as is shown in Plates XIII and XIV and in cuts C and D, but as the cells break apart and become free they wander into all parts of the body cavity, and all traces of the somatic layer as a distinct tissue soon disappear, although, as shown in Plate XIII, Figs. 3, 4, 5 and 6, its outline is preserved for a short time after the cells are completely separated. The body cavity, which appears to be empty, is undoubtedly filled with a fluid or semi-fluid serum, of greater density than water, and sufficiently firm to hold the amœboid cells and to keep them floating. By the time that the pharynx appears, Plate XIV and Plate XXII, the somatic layer is completely broken down. Plate XLII, Fig. 11, is part of a transverse section across the middle line of the dorsal surface of an embryo at the time when the last remnant of the somatic layer is going to pieces, and it shows the way this takes place. In this figure *21* and *22* are the folds of the embryo sac, *b'* is the epithelial capsule, *a* is the ectodermal rudiment, *15* is the body cavity, and *8* the follicle cells. These are multiplying rapidly by direct division of the nuclei, and their exposed ends are irregular and amœboid. Many of the cells break away bodily, while others divide into a portion which retains its position, and a portion which splits off as a free amœboid cell. Multiplication of the nuclei goes on in the detached cells, as well as in those which are fixed, and two nuclei are usually present in most of the wandering cells.

Thus the embryonic portion of the somatic layer of follicle cells disappears as a distinct layer, and at the stage shown in Plates XVI and XVII, and in Fig. 2 of Plate XLI, it is represented only by migratory amœboid cells scattered through the body cavity of the embryo.

The next part of the follicle to disappear is the somatic lining of the perithoracic system. As shown in Plate XLII, Figs. 6 and 7, the cells of this layer become detached and acquire amœboid outlines before the gill-slits acquire their openings into the pharynx, and as I have already stated, all of them ultimately separate and pass into the cavity of the cloaca, which becomes so well filled with them that at the stage shown in Plate XVIII, Fig. 8, the outline of the cloaca is hard to trace in the sections. The details of the process of disintegration, which are shown in Plate XLII, Fig. 8, g'', are so much like the history of the somatic layer just described, that no further account seems necessary. The disintegration of the visceral mass of follicle cells begins with the formation of the cavity of the pharynx, as already described and figured at c in Fig. 8. After the blastodermic epithelium of the pharynx is formed, most of the visceral follicle cells are left outside it in the body cavity, as shown at 8 in Plate XVII, Figs. 1, 2 and 3, where they form a secondary wall outside the blastodermic epithelium, as they do also around the gill-slits, Plate XVI, Fig. 3, and the cloaca, Fig. 2. All these cells ultimately degenerate and disappear, but while most of them first become migratory and amœboid, as shown at 8 in Fig. 9 of Plate XLII, others become vacuolated and break down and disappear without any migration, as for example those which are shown in Plate XVII, Fig. 6, included between the epithelium of the cloaca, g''' and the ectoderm a, at the point where the cloacal aperture is to be formed. The history of the process of degeneration is as follows: The large irregular amœboid follicle cells, usually with two nuclei, Plate XX, Fig. 5, wander into all parts of the body cavity and become very much vacuolated, and while this is going on the small amœboid mesoderm cells of the embryo lodge upon their surfaces, as they do upon the surfaces of the embryonic cells, and give rise to a fibrous capsule around each follicle cell or group of cells, as is shown in Plate XX, Figs. 5 and 6, Plate XXI, Figs. 1 and 2, and Plate XIX, Figs. 9 and 10. Mesoderm cells or blood corpuscles are often found also in the vacuoles of degenerating follicle cells, as in Plate XX, Fig. 5. As the cells disappear they leave behind them an empty meshwork of fibers, as is shown in Plate XXI, Fig. 2.

While it is of course impossible to trace the history of each follicle cell, I think there is sufficient evidence to warrant the statement that, while the organization of the embryo is at first blocked out in follicle cells, all of this scaffolding is afterwards torn down; for I have found follicle cells in the act of detachment in all the regions of the embryo and in all the follicular structures.

Section 7.—*The Placenta.*

We have now to trace the history of the placenta, and as this organ is much simpler in Salpa hexagona than it is in Salpa pinnata, I shall first describe the origin and fate of the placenta of Salpa hexagona.

The part of the follicle which is bathed by the blood of the chain-salpa begins to grow, Plate XI, Fig. 3, as soon as the rest of the surface of the embryo is covered by the epithelial capsule, and it soon becomes many cells thick, and irregular folds and spaces appear in it. As soon as the walls of the placental chamber, Plate XLV, Fig. 1, are formed by the supporting ring, *23*, the thickened portion of the follicle grows down into it as a ring, *31*, around its sides, and as a free pendant mass, *24*, which Barrois has compared to a bell-clapper. As the embryo grows, and the cavity of the placenta becomes larger, Plate XLV, Figs. 2, 3 and 4, cell multiplication goes on very rapidly in these structures, until the chamber is completely filled by convoluted inosculating strings of cells, so arranged as to break up the blood space into a number of tortuous channels, in which the circulation is retarded, so that the plasma and the blood corpuscles are almost brought to rest, as they find their way through these obstructed passages. Figs. 2, 3 and 4 of Plate XLV are sections through the placenta and embryo of Salpa hexagona at three successive stages of development, but they will also serve to illustrate the structure of the placenta, since they cut it in three different planes. Figure 4 is from an embryo at the stage of Plate III, Fig. 4, passing through the neck of the placenta and through the gill, *o*. Comparison with Plate III, Fig. 4, will show that it is a vertical transverse section in the vertical axis of the placenta. Figure 3 of Plate XLV passes through the neck of the placenta and through the ganglion, *s*, and while it is from an embryo considerably younger than Fig. 4 of Plate III, comparison with this figure will show that it must cut the greater part of the placenta in front of the median plane of Fig. 4. Fig. 2 is from a still younger embryo, passing through the neck of the placenta, and through the place of the cloacal aperture, *g*ᵛ. At this stage this point is very much farther forward than it is in the embryo shown in the figure on Plate III, and nearly where it is shown at *g*ᵛ in the embryo of Salpa pinnata shown in Fig. 3 of Plate XLI, but examination will show that the section must cut the greater part of the mass of the placenta about as much behind the plane of Fig. 4 as the section in Fig. 3 is in front of it. While all these sections pass through the neck of the placenta, the upper part of Fig. 3 is in effect a vertical

transverse section through the anterior portion, Fig. 4 through the middle portion, and Fig. 2 through the posterior portion of the body of the placenta. The cellular strings become more and more numerous and crowded as the placenta grows, as is shown by the series of stages, but they are always most developed in the middle plane, as is shown in Fig. 4, while in front and behind the spaces are much larger, and the cellular strings more independent. At the stage shown in Fig. 1 the blood circulates vaguely back and forth into and out of the placenta, but horizontal sections show that at the stages of Figs. 2, 3 and 4 the neck of the placenta is divided into two openings, an anterior and a posterior, by a median transverse partition. This partition is not flat, but folded in such a way that no single vertical section can show the whole of it, but each series shows it at all positions between that of Fig. 2, where it is united below the neck of the placenta to the wall of the cloaca on the left, to Fig. 3 where it joins it on the right.

The partition is continuous with the " blood-bud," *24*, and is formed from a substance which appears to be the same as the cellulose mantle. This gelatinous substance is formed in many parts of the body of salpa, between the mesodermic endothelium of the body cavity and the endodermal structures.

It is shown, for example, at *32* in Figs. 3 and 5 of Plate XXXIV. This partition divides the cavity of the placenta into an anterior blood chamber, Plate XLV, Fig. 3, *bl, 1*, and a posterior chamber, Fig. 2, *bl, 2*, which communicate with each other through the spongy mass formed by the plexus of strings of cells which fills the middle portion, as shown in Fig. 4.

In Salpa pinnata the partition is formed by the "blood-bud," *23*, itself, which in advanced embryos fits like a plug into the neck of the placenta in transverse section, Plate XVIII, Fig. 4, while in longitudinal section, Fig. 8, or in a transverse section in front of the plug, Fig. 2, or behind it, Fig. 5, there is a free channel for the entrance or exit of blood, as is shown also in Plate XXXV, and in the series of horizontal sections from the same stage shown in Plate XLVI, Figs. 2, 3 and 4. The blood which enters the placenta behind this partition and passes into the chamber marked *bl, 2*, must make its way slowly through the spongy mass of cells before it can gain access to the anterior chamber, *bl, 1*, and escape from the placenta, and the conditions are of course essentially the same when the circulation is reversed. The general anatomical structure of the placenta of Salpa pinnata can be understood by comparing the

longitudinal section in Plate XXXV with the transverse sections in Plate XVIII, and the horizontal sections in Plate XLVI, Figs. 2, 3 and 4. In a horizontal section near the top, Fig. 2, the cavity is cut up by strings and clumps of cells which have no constant arrangement, although the study of sections at a lower level shows that certain channels, *bl, 1* and *bl, 2,* communicate with one or the other of the two chambers which open into the blood system of the chain-salpa, while others are channels of communication between one of these chambers and the other. At a somewhat lower level, Fig. 3, the strings of cells gradually unite to form a transverse partition which separates the anterior chamber, *bl, 1,* from the posterior, *bl, 2,* and these chambers gradually become more and more capacious, and the blood channels in the partition less and less numerous, until, near the neck of the placenta, Fig. 4, the partition becomes, in longitudinal sections, a straight rod, running across the placenta like the handle of a basket.

In a section just above the neck of the placenta the ends of this rod are separated from the supporting ring by a space, *33,* which is also shown at *33* in Plate XXXV. This space runs around the whole circumference of the placenta, as shown in Fig. 4, and it is bounded internally by the endothelium of the placenta and externally by the supporting ring. It is interrupted at short intervals by fibers which cross it, and it is lined by very small cells, very much smaller than the blood corpuscles. This space acts, perhaps, as a valve to close or expand the opening of the placenta, although sections of preserved specimens throw little light on its function. The strings of cells are nourished by the plasma of the blood which is retarded in the meshes of the spongy meshwork, and also by the blood corpuscles; and in both Salpa pinnata and Salpa hexagona, blood corpuscles may be found settling upon the surfaces of the strings and sinking into their substance, as is shown in Fig. 5 of Plate XLVI, which is a highly magnified drawing of part of Fig. 3.

SECTION 8.—*The Nutrition of the Embryo.*

As the mammalian placenta nourishes and aerates the blood of the fœtus by the diffusion of gases and food in solution through the walls of the blood-vessels, it has been generally taken for granted that the placenta of salpa performs its function in the same way, and it has been described as divided into a fœtal chamber and a maternal chamber, although its cavity is in reality part of the body cavity of the chain-salpa, and the blood which circulates in it that of the chain-salpa. The salpa

embryo is bathed by the water which is constantly flowing past it, and it is therefore in very much closer relation to the external world than a mammalian embryo, shut up in the interior of a large thick-walled body. There does not seem to be any need in salpa for a respiratory placenta, and its thick spongy walls seem to indicate that it is not respiratory. We find in its structure nothing like the interlacing villi of the mammalian chorion, and the sections show that the embryo is nourished in a way quite unlike anything which has been described in the mammalia.

The subject is a very interesting one. The rapid growth of the salpa embryo is one of its most conspicuous characteristics, and the nutrition which this rapid growth demands is secured by two very peculiar organs, the follicle and the placenta.

While the egg at the time of fertilization is very minute, the embryo at the time of birth is enormous, as compared with the size of the chain-salpa which carries it, and it certainly increases many thousandfold during development. The growth is only partially due to cell multiplication, and it is in part a result of the growth of the individual cells, for instead of growing smaller with repeated division, they actually increase in size in all parts of the body.

As the older stages are less magnified in the figures than the younger ones, this growth of the cells is not conspicuous in the figures, but it is one of the most notable peculiarities of the salpa embryo, and in many parts of its body cells as large as the original ovum are found. The growth sets in very early, and it goes on uninterruptedly throughout the whole fœtal life, so that the embryo becomes gigantic as compared with the body of the chain-salpa which contains it. Quoy and Gaimard describe an embryo, two inches long at birth, in a salpa (S. forskalii) a foot long, and Leuckart says that the embryo of S. democratica at birth is two-fifths as long as the chain-salpa which carries it. The fully grown embryo of S. hexagona is almost as long in comparison with the chain form of the same species.

It is not unusual for the embryos of viviparous animals to gain slightly in size and weight before birth, but, as Leuckart points out, the mammals are the only animals which exhibit anything comparable to the rapid growth of the salpa embryo from a minute egg, and the history of the salpa embryo at once calls to mind the growth of the embryo in the placental mammals; nor is this resemblance entirely superficial, for in both the mammal and in salpa we find an especial fœtal organ, the

placenta, for the purpose of affording to the growing embryo an abundant supply of nutriment.

The resemblance between the fœtal life of salpa and that of a mammal is most remarkable, and it is all the more noteworthy since we may be absolutely confident that the placenta of salpa is an independent acquisition, entirely without genetic relation to that of mammals.

No modern writer except Todarro has ventured to regard the two structures as homologous, and their phylogenetic independence is so obvious that it is not necessary to discuss it, although a greater physiological and anatomical resemblance than the facts warrant has usually been assumed.

We should hardly expect fundamental similarity in structures of diverse origin. On the contrary, we might reasonably look for profound differences between the placenta of salpa and that of the mammals.

The various writers on salpa, while recognizing this fact, and while pointing out the great differences in the way in which the placenta is formed in the two cases, have nevertheless assumed, either explicitly or by implication, a much greater resemblance to the mammalian placenta, in structure and in function, than actually exists. The later writers say very little about the function of the placenta of salpa, but they assume a fundamental similarity to its function in mammals.

So far as it is in both cases an organ for supplying the embryo with nutritive matter, derived from the blood of the supporting organism, the resemblance is real, but it goes no farther than this, and the way in which the nourishment is conveyed to the embryo is totally unlike; a fact which has never been described nor even noted.

In the mammalian placenta the blood of the embryo, as it circulates through the villi of the chorion, is brought into such close contact with the blood of the mother, that diffusion takes place through the separating walls, and thus the blood of the fœtus is oxidized, relieved of its waste products, and supplied by diffusion with nutritive matter in solution.

Notwithstanding the very intimate union between the blood-vessels of the fœtus and those of the mother, there is no direct communication between them, and nothing except gases and liquids can pass from the body of the parent to the body of the child, without the violent rupture or perforation of the walls of the vessels, unless, perhaps, some very minute bacteria are an exception.

It has been generally assumed that this must be true of salpa also. Thus Barrois says, incidentally and very briefly (4) p. 495, that the func-

tion of the placenta of salpa is to bring about by osmosis an interchange of fluids between the blood of the parent and that of the embryo, as in the placenta of a mammal.

The subject has received very little attention, but as no one has ever commented upon the view set forth at considerable length by Leuckart (1) pp. 61 and 62, this may be regarded as the accepted view. He says: "The histological differentiation of the organs and tissues of the embryo is accelerated, to a high degree, by the circulation in the body of the young salpa, which is completely separated from the circulation of the mother. At no time does the blood of the mother pass through the wall of the placenta into the body of the embryo. The transfusion between the mother and the fœtus is, as in the mammals, purely endosmotic, through the substance of the placenta, and it is most essentially facilitated by the movement of the blood, both in the embryo and in the chain-salpa.

"The upper wall of the placenta, which is the peculiar seat of the process of diffusion, projects into the body of the embryo, and is surrounded by the median ventral blood sinus. As the blood corpuscles of the embryo are much smaller than those of the chain-salpa, it is easy to see that no mingling takes place."

It is probably true that no transfusion of blood corpuscles takes place, and it is difficult to show from the study of sections of hardened specimens that no serum from the blood of the chain-salpa is diffused through the wall of the placenta, although its great thickness seems to be a very unfavorable condition for this purpose, and I shall show farther on that the mechanism of nutrition is very different from that of mammals; that this is effected by the actual migration of great placenta cells, Plate XLV, Fig. 4, 29, into the body cavity of the embryo. The placenta is an organ for the nourishment of the placenta cells by the blood of the chain-salpa; and the subsequent degeneration of these cells, after they have migrated into the body of the embryo, supplies the material for the growth of the embryo. This is in all probability the only function of the placenta, for there does not seem to be any need for an especial apparatus for oxidation, or for the removal of waste products. The salpa embryo stands in much more direct relation to the external world than the mammalian embryo. It projects into the cloaca of the chain-salpa, and is freely exposed to the constant current of fresh sea-water which flows around it, and its thin surface seems to be much more favorable than the thick wall of the placenta for the diffusion of gases.

During the later stages of fœtal life its own mouth is open, its muscles contract, and there is no reason why it should not breathe for itself exactly like an adult. I therefore regard the placenta as a nutritive organ, pure and simple, and it serves its purpose not by the diffusion of a fluid, but by the transportation of solid food into the body of the embryo. From this point of view it is clear that those investigators who have described it as divided into a fœtal chamber and a maternal chamber have been misled by an erroneous notion of its function.

The detachment of the placenta cells has been observed and noted by both Salensky and Barrois, but it has been regarded as a destructive change and as a sign that the organ has served its purpose and has become superfluous.

It has been assumed that it reaches its perfect form and serves its purpose, and that it then degenerates and breaks down, and no importance has been attached to the process of degeneration, as it has not been regarded as significant.

No note has been made of the very early stage at which degeneration begins, nor of the fact that it is initiated as soon as the embryo begins to grow, and long before it has reached half or a quarter of the size which it is to have at birth.

This is hard to explain so long as the disintegration of the placenta is regarded as its destruction, but it becomes quite intelligible as soon as we learn that the detachment of the placenta cells, instead of marking the end of its functional life, is actually a manifestation of its useful activity.

As the figures in Plate XLV show, the strings of cells multiply at their lower ends by direct division of their nuclei, and as the new cells which are thus formed push up towards the top, they grow very large, while their nuclei become filled with diffused chromatin granules. In Salpa hexagona these cells ultimately reach the top of the placenta, where they gradually become elongated and irregular, and then break through into the body cavity of the embryo as the migratory follicle cells which are shown at *29* in the figures.

While the details are slightly different in Salpa pinnata, placenta cells migrate bodily into the embryo in the same way, and they are shown in many of the figures, as in Plate XVIII, *29*, for example.

The rapid growth of the embryo seems to be most important to salpa, and while we know almost nothing of its birth rate, the quickness with which the surface of the ocean becomes covered with salpæ of all

ages in a long calm, shows that the animals are most prolific, and the complicated structure of the organs for nourishing the embryo shows that every provision is made for rapid growth.

The placenta is not the only nutritive organ, for, as we have seen, the follicle also makes most important contributions to the supply of material which is available for the construction and rapid completion of the body of the embryo, and while I have spoken of the segmentation and the formation of the blastodermic germ layers as retarded, the retardation is probably not actual, but only relative, and the process of development is, on the whole, accelerated by the presence of the follicle, and by its share in the growth of the embryo.

I have now shown that the ultimate fate of all the follicle cells is the same, and that they may be found, in the sections, detaching themselves and degenerating, first, in the somatic layer of the embryo; secondly, in the somatic follicular lining of the perithoracic structures; third, in the cavity of the pharynx; fourth, in the visceral mass outside the digestive cavity, and last, in that part of the placenta which is derived from the somatic layer of the follicle.

While it is not possible to trace the history of every cell from first to last, we have as ample evidence as we could hope from sections, that the function of the follicle of salpa is exclusively nutritive; that it is transitory and embryonic, and that the tissues of the embryo are not built up out of follicle cells, but from blastomeres, after the analogy of all the rest of the animal kingdom.

CHAPTER III.

THE MORPHOLOGICAL SIGNIFICANCE OF THE SALPA EMBRYO.

A basis for the comparison of the salpa embryo with even its closest allies is hard to find, for although it is still a true embryo and not a bud, its early stages have been profoundly modified by secondary changes.

Salensky holds indeed, (5) 396, that a knowledge of the development of other animals does not help to clear up the obscurity which involves the salpa embryo; that its peculiarities are so different from all that we know of other animals that we must not hope to bring it into the general scheme of animal embryology, and that, while it begins its development by the sexual method, this soon gives place to budding from the wall of the follicle.

I have shown that this view is untenable, and that the embryology of salpa is not totally and fundamentally irreconcilable with the principles of general embryology, although it is quite true that the nature and origin of the secondary changes are most perplexing subjects.

As our starting-point in making comparisons, we may safely assume that the fundamental plan of development was originally that of the Tunicates in general, but we have very few facts to show the way in which the complications were introduced. Salensky believes that the embryology of Salpa bicaudata is less modified than that of the other species, but only as regards accessory structures. As regards the peculiar history of the blastomeres and migratory follicle cells this species is like the others, and there is, so far as we know, no species which presents a transitional stage in this history. Nor can we get much help from Pyrosoma or Doliolum, the two nearest allies of Salpa, for while the life-history of Doliolum, with its invaginated gastrula and its tailed larva, is possibly more primitive than that of Salpa or Pyrosoma, it presents no trace of the distinctive peculiarities of either of them, and it therefore affords no better basis for comparison than the ordinary Tunicates.

The embryos of Salpa and Pyrosoma represent two distinct lines of secondary modification, and neither serves as an explanation of the other.

We therefore have only a slight basis for a phylogenetic history of the modifications which the salpa embryo has undergone in reaching its present form.

We must remember, though, that it is possible to make instructive and valuable comparisons even when they do not lead to exact or definite phylogenetic conclusions, as when we compare adult echinoderms with each other without committing ourselves to any view of their ancestral relationship; and I think we may give clearness and definiteness to our conception of the salpa embryo by comparing it with the embryos of other Tunicates, although we may not be able to mark out the path it has followed in reaching its present structure. We cannot have a clear notion of structure without comparison, although we may make noteworthy progress without historical data.

Even if we were totally without evidence as to the history of the secondary modifications which the salpa embryo has undergone, their morphological nature could be studied by comparing it with the embryos of other Tunicates, and I shall show that we do have some evidence, although it is true that this is scanty.

The Embryology of Primitive Tunicates.

The embryology of Pyrosoma, the nearest relative of Salpa, is complicated by the early degeneration of the embryo, and by the early appearance of asexual multiplication by buds. Both these peculiarities are secondary, and they have been acquired, or at least very much accented since Pyrosoma and Salpa diverged from their common ancestor. We may therefore leave the four Ascidiozooids of the Pyrosoma embryo out of consideration, and take the Cyathozooid as a basis for comparison with the salpa embryo, but we must do away in imagination with the degeneracy of the Cyathozooid, and must picture it as an embryo with the potency of an adult, like the embryo which becomes a solitary salpa.

We may also assume that, at some time in the past, these embryos, like the embryo of Doliolum, passed through an Appendicularia-like larval stage, corresponding to the tadpole larva of other Tunicates. Still further back, we must believe that at some remote period the ancestral embryo of all the Tunicates and of all other chordata passed through an invaginated gastrula stage, formed by the total regular segmentation of a holoblastic egg. We are also forced, by all the facts of embryology, to

believe that the whole organization of the body, in the most complete form which it had then attained, was latent or potential in the fertilized egg, and was formed from it by cell multiplication.

Furthermore, we may be sure that, for a time, all the chordata originally followed the same line in their ontogenetic development from the egg; that all the Tunicates retained a common life-history for a longer time; that the embryos of Salpa, Pyrosoma and Doliolum were originally alike for a little longer, and that the differences between the embryos of these three forms are the results of more recent modification.

These statements involve no opinion on the exact character of the relationship between the ordinary Tunicates on the one hand, and Salpa, Pyrosoma and Doliolum on the other, and no opinion on this point is necessary for our purpose, which is to study the morphological significance of the salpa embryo.

Has the Egg of Salpa passed through a Stage with a Large Food-Yolk?

Pyrosoma has a big food-yolk and a disk-shaped blastoderm; and the arrangement of the germ layers and the anatomical relations of the embryo are profoundly modified by its presence. Was this yolk acquired before or after Pyrosoma and Salpa diverged from each other?

The salpa egg has nothing of the sort now, and it undergoes total segmentation, but if the yolk was acquired before the divergence of the two life-histories, we must, in our comparative study of the salpa embryo, take into consideration the inherited results of its existence in the ancestors of this embryo.

The question cannot be definitely answered, but the incompleteness of the ectoderm and the slow closure of the floor of the pharynx of salpa are what we might expect if a food-yolk has once been present. In an ordinary gastrula the continuity of the germ layers is complete, but it will be seen by comparing the figures in Plates XVI, XVII, XVIII and Figure 11 of Plate XLII, that the ectoderm of salpa has a growing edge, and that it gradually spreads out on all sides over the embryo until its growth is stopped, in the older stages, by the placenta. The starting-point from which the ectoderm spreads is on the middle line of the dorsal surface of the embryo around the region which I shall soon give my reasons for regarding as the place of the blastopore.

In meroblastic eggs, like those, for example, of Teleosts and Birds, it is well known that even after the germ layers are established they have

an external or peripheral boundary and a growing edge, and that the inclusion of the yolk is a very slow process.

From Salensky's statement (17), p. 455, that "outside the limits of the germinal area the yolk is perfectly naked," I infer that in Pyrosoma it never becomes completely inclosed by the ectoderm of the cyathozooid, but that this degenerates before the yolk is covered. This secondary adaptation to the presence of a food-yolk is so well understood that we need not dwell on it. So far as I am aware, the salpa embryo is the only one without a food-yolk which is known to have an incomplete ectoderm with a growing edge, and the incompleteness of the floor of the digestive tract is another fact of exactly the same kind. There is therefore good reason for believing that salpa is descended from a form with a food-yolk, like that of Pyrosoma, which has afterwards been lost. The acquisition of new methods of nourishing the embryo by means of migratory follicle cells and a placenta is a satisfactory explanation of the way in which the need for a food-yolk was done away with, and its disappearance is therefore quite intelligible.

In considering the influence of a food-yolk upon the structure of the embryo, we must have a clear idea of what the books call its "morphological position." To my mind, the way this term is used by writers on the embryology of vertebrates is open to criticism. The fact that the unconsumed remnant of the yolk of vertebrates is surrounded by endoderm or inclosed in endoderm cells, is no evidence that it was laid down in the egg in any relation to the regions of the body of the future embryo, or in any "morphological position" whatever. The series of stages in the phylogenetic history of the acquisition of a food-yolk is a series of eggs, not embryos. In secreting the yolk the egg functions as a cell, not as a potential embryo, and there is no reason to believe that the yolk material is laid down in any definite relation to the structure of the latent embryo. Its distribution is probably determined by the structure of the egg as a cell, and the place which the unassimilated remnant occupies in the body of the embryo depends upon the physiological activity of cells of the embryo itself. In fact, as all the cells of a young amphibian embryo are packed with yolk, the yolk of an amphibian egg has no "morphological position." The yolk of Pyrosoma is not inherited from a common source with that of true vertebrates, and there is, of course, no reason why it should be assimilated in exactly the same way. I believe that a careful study of Salensky's figures will show that it is in the body cavity, and not in the digestive cavity of the embryo, for no layer of endoderm ever extends over any considerable part of it.

If the salpa embryo has ever had a food-yolk it undoubtedly agreed in all respects with that of Pyrosoma and lay in the body cavity.

It may, perhaps, seem to some that the incompleteness of the germ layers of salpa may have been acquired as an adaptation to the presence of the follicle or the placenta, and that it may therefore be quite independent of the same phenomenon in Pyrosoma, but this view cannot be seriously urged, for while we may believe that the ectoderm is potentially present, in an undifferentiated state, in the surface of the yolk outside the growing edge, as it certainly is in young amphibian embryos, we cannot believe that it is potentially present in the follicle of salpa.

I therefore think that we may safely assume, from the incompleteness of the germ layers of Salpa, that its ancestors had a food-yolk like that of Pyrosoma.

The Primitive Salpa Embryo.

Whatever view of the food-yolk we take, there can be no doubt whatever that, far back in the past, the ancestor of both Salpa and Pyrosoma had simple holoblastic eggs like those of Clavelina. This is a necessary deduction from the principles of comparative embryology, and it is also supported by the fact already pointed out that the salpa egg shows the same type of segmentation as that of Clavelina.

We may therefore safely assume, as the point of departure for our comparative study of the embryo, a life-history like that of the primitive chordata, where the holoblastic egg undergoes total regular segmentation and gives rise to a hollow blastula, from which a gastrula is formed by invagination.

From the dorsal part of the endodermal wall of the primitive digestive cavity the notochord was formed, and the nerve tube arose from the dorsal median ectoderm. At least one pair of ectodermal ingrowths united with paired endodermal outgrowths from the pharynx, to form the pharyngeal clifts through which the digestive cavity opened to the exterior. The region of the tail elongated and the embryo became an active locomotor chordate animal. So far the characters of the primitive salpa embryo are common to the chordata.

Furthermore we must assume that in the larval stage of all known Tunicates, except Appendicularia, the ectodermal portions of the gill-tubes moved towards the middle line, and united to form a common dorsal cloaca, into which the digestive and reproductive organs came to open.

The embryology of Salpa furnishes, as I shall show in another section, still further evidence regarding the phylogeny of the adult, but we are here concerned only with the comparative study of the embryo.

The Origin and Significance of the Follicle of Salpa.

The most peculiar and anomalous feature in the life-history of Salpa is the follicle, but even here we are not absolutely without means for comparative study.

In numerous, widely separated members of the animal kingdom, follicle cells migrate into the substance of the ovarian eggs, and supply them with food. Many instances are found among the Tunicates; Clavelina for example; and, as we have seen, Salpa must be added to the list. Salpa undoubtedly inherits this peculiarity, which is also found in Doliolum, from their common ancestor, and very probably from a still more remote ancestor, common to the other Tunicates and to Appendicularia.

Kowalevsky (Arch. f. Mikros. Anat., Band XI) was the first to show that the migration and degeneration of follicle cells goes on in Pyrosoma after the egg is fertilized. He says that great numbers of follicle cells separate from the follicular epithelium and wander into the space between it and the developing egg, as free inner follicle cells. Some of them gather in groups external to the germinal disk, while others work their way in between the segments and are for a long time distinguishable from the blastomeres. Finally, he says (p. 607), these cells as well as the·yolk are surrounded and inclosed by the blastoderm and turned to nutritive material or to blood corpuscles.

While Salensky believes that instead of nourishing the embryo these migratory follicle cells take part in its construction, his account of their origin is essentially like Kowalevsky's, except that he shows that besides those which make their way between the blastomeres, many others pass under the edge of the blastoderm and accumulate in the segmentation cavity, or the space between the blastoderm and the yolk, and that others wander into the substance of the yolk.

Cuts E and F are two early stages in the segmentation of Pyrosoma, copied from Salensky's memoir. They are sections through the blastoderm, and the food-yolk is not represented. It should occupy the space below the figures. In my pen-copy of his figures I have dotted the migratory follicle cells to make them more conspicuous. In cut E they

are shown migrating into the segmentation cavity under the edge of the blastoderm, and filling the space between it and the yolk; while in cut F they are shown pushing in between the blastomeres, and some of them are embedded in their substance.
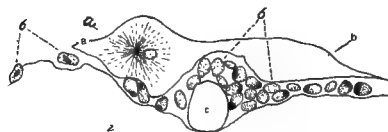
CUT E.
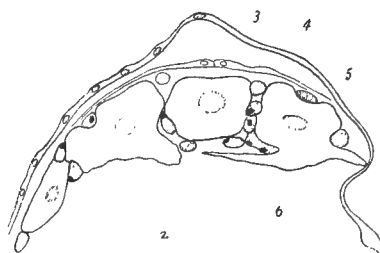
CUT F.

In Salpa there are two periods of migration. One, shown in Plate XXXI, Figs. 5 and 6, which has never before been described, is very early in the history of the ovarian eggs, when the migratory cells are assimilated by the egg cell, and thus furnish the material for its growth. A second period of migration, which was discovered and minutely described by Salensky (5), begins immediately after fertilization, and the migration goes on for a long time with great energy and rapidity on an extensive scale, as shown in my Plates IX and X.

Pyrosoma shows this migration of the follicle cells into the substance of the embryo in a simpler form than Salpa, and while there may be no conclusive evidence that the life-history of Salpa has come about by the modification of that of Pyrosoma, there can be no doubt that the two have had a common starting-point, and that, so far as the migration of follicle cells goes, the Pyrosoma embryo is nearer this starting-point than the Salpa embryo. It may therefore be used to interpret the structure of the salpa embryo, and I shall attempt this without assuming that the Pyrosoma embryo is in the direct line of modification.

We have earlier stages in the evolution of the migratory follicle than that exhibited by the Pyrosoma embryo. In cuts G and H, I have copied from Davidoff two figures of early stages in the segmentation of Distaplia magni larva. Cut G is an egg which has divided into two blastomeres, and cut H is one in which the ectoderm and endoderm are differentiated.

The egg is surrounded by a capsule of follicle cells, 6, which are equivalent in ultimate origin to the germ cells, as they are in Salpa. In Distaplia, according to Davidoff, the layer of cells by which the growing egg cell becomes surrounded are so directly derived from cells which are destined to become eggs, that this author calls them abortive eggs.

During the early stages of segmentation some of the cells which surround the egg of Distaplia pass into the fissures between the blastomeres (p. 536) in much the same way that they do in Pyrosoma, but on a much less extensive scale.

Ultimately, after segmentation is well advanced, (p. 548), these cells enter into the substance of the endodermal blastomeres, and Davidoff has no doubt (p. 549) that they serve as food for the endoderm cells.
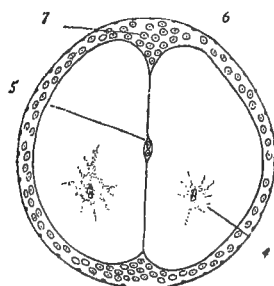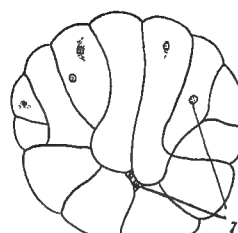
CUT G.　　　　　　　　CUT H.

In cut G the outer follicle, *6*, is separated from the surface of the egg by an intervening space, which is filled with free follicle cells, *7*, or, as the author calls them, abortive eggs, which have migrated into it from the capsule. As the blastomeres are marked off by segmentation these cells wander into the spaces between them, where some of them are shown at *5*. Except for these spaces filled by migrating cells, the blastomeres are in contact, and there is no empty segmentation cavity. As development goes on the migrating cells penetrate the substance of the blastomeres, as shown in cut H, and are used up as food, so that at last all traces of the follicle disappear. In Distaplia the blastomeres are so much larger than the migratory cells, at the latest stage when these are found, that the two sorts of cells are easily distinguishable, and it is certain that the follicle cells take only a nutritive part in the construction of the embryo.

In Pyrosoma and in Salpa, most of the migratory cells retain their individuality until the blastomeres have become so small by repeated division that they are no larger than the follicle cells, and it becomes very hard to follow the course of each cell and to trace its fate. I have shown that while this is difficult in Salpa pinnata, it is not impossible, and that the ultimate fate of all the follicle cells is to supply food for the cells of the embryo. In the embryo of Pyrosoma, Salensky has traced the history of the two sorts of cells until they are equal in size and no

longer distinguishable from each other, and as he finds no cells in the act of degenerating, he believes that they all help to build up the embryo; but there is every reason to believe that the follicle cells have the same fate in Pyrosoma that they have in Distaplia and Salpa.

While the Distaplia egg undergoes total segmentation, it is well filled with yolk, and the segmentation cavity is thus obliterated; but this is a secondary change, and we may picture the egg without it, as we may also, in imagination, divest the Pyrosoma embryo and the·Salpa embryo of all secondary peculiarities except those which engage our attention; and the most satisfactory way to study the history of the follicle in these three embryos is to strip them mentally of their irrelevant peculiarities and picture them as reduced to their simplest expression, and to imagine them as developing according to the primitive chordate type. I have attempted to do this in the following diagrams.

If we picture the Distaplia egg without its yolk, and with a spacious segmentation cavity, it will be like cut I, in which *15* is the segmentation cavity and *2* is the epithelial capsule of follicle cells. The space between this and the embryo is filled with migratory cells, and these are represented as wandering into the substance of the blastomeres, and also pushing their way between them into the segmentation cavity.

In Distaplia the follicle cells are used up very early, but I have represented them in the diagram as persisting in great abundance at an advanced stage of segmentation, as is the case in Pyrosoma and Salpa. The blastula in the diagram is figured as about to undergo invagination to form a gastrula, and the cells in the left hemisphere, *3*, are ectodermal, while those in the right, *7*, are endodermal. For reasons drawn from the embryology of Salpa, I have represented the migration into the substance of the blastoderm cells as most active in the endodermal hemisphere, and the migration into the segmentation cavity as most active in the equatorial zone where the two germ layers meet, and as more active between the endoderm cells than between the ectoderm cells.

If now we picture the invagination of a blastosphere like cut I, we have a gastrula like cut J. As this stage is more advanced than the first, the epithelial capsule of the follicle is represented as entirely broken up into distinct cells, and while a few of these are still outside the embryo, most of those which have not migrated into its substance are folded into the digestive cavity as this is formed. A few of the migrating cells are in the ectoderm, more in the endoderm, and others are pushing in between the cells, especially around the edge of the blastopore, and wandering

into the segmentation cavity or body cavity. In Salpa these latter become arranged in an epithelium or continuous layer, and in cut J, I have represented them as beginning to assume this structure by settling down over the inner ends of the blastoderm cells.

The embryo of Pyrosoma is a disk-shaped blastoderm on a surface of food-yolk; but so far as the migratory follicle cells are concerned, I regard it as comparable to cuts I and J; and if we imagine the gastrula in cut J converted into a disk-shaped embryo on the surface of a big yolk, we shall have something like the Pyrosoma embryo, so far at least as the follicle cells are concerned.
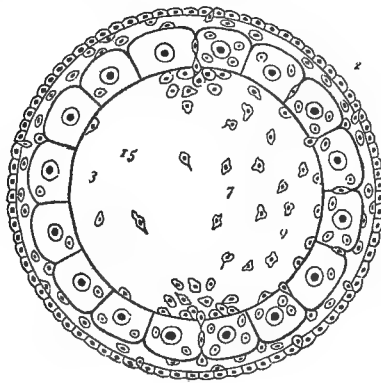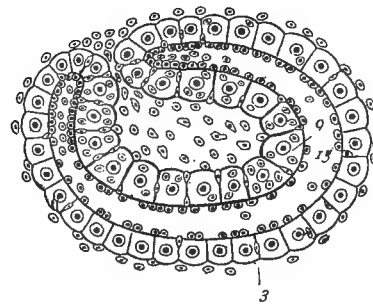
Cut I.　　　　　　Cut J.

We do not know whether the migratory habit of the follicle cells and their peculiar share in the development of many Tunicates were or were not acquired before the embryos departed from the primitive type which is shown in these diagrams; but however this may be, we know that they were acquired by embryos which were, at least, modifications of this type, and we may therefore refer them back to it in imagination and picture them to ourselves as added on to it.

Between the Pyrosoma embryo and Salpa embryo there is a gap which may be filled, in imagination, in this way. Suppose the follicle cells, which, in cut J, lie in the body cavity of the gastrula, to become arranged over the inner ends of the cells of the ectoderm and endoderm, so as to form a continuous epithelium of elongated cells, as shown in cut K, where *3* is the ectoderm, *9* the endoderm, *15* the body cavity, and *7* and *8* the two layers of follicle cells; and suppose also that besides these follicle cells there are others, in the substance of the endoderm cells, and others in the digestive cavity, and a few outside the embryo in the vicinity of the blastopore. In Plate X, Fig. 8, the follicle cells, which are colored blue, have very nearly the arrangement that they have in

cut K, although the salpa embryo exhibits other peculiarities which have not yet been discussed. In the diagram the arrangement of the follicle cells inside the body cavity is so much like the arrangement of a mesoderm that I have called its outer and inner layers somatic and visceral, after the analogy of the mesoderm. At the stage shown in the diagram the embryo has a body wall made up of an outer layer of ectoderm, 3, and an inner layer of somatic follicle cells, 7; and a digestive cavity whose wall is made up of a layer of endoderm, 9, and a visceral layer of follicle cells, 8, while its lumen is filled with follicle cells. The body cavity is bounded on all sides by follicular epithelium.
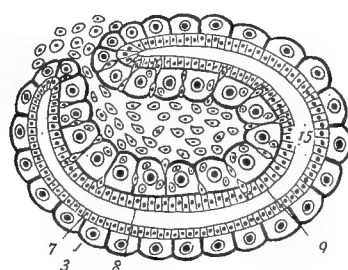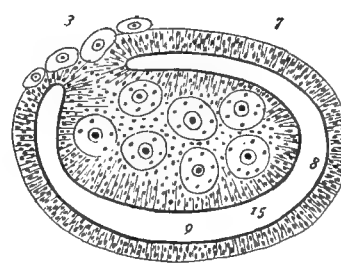
CUT K.   CUT L.

Now if we picture this gastrula as gradually shaping itself into a Tunicate embryo, it is plain that, as the various organs are blocked out by foldings and ingrowths and outgrowths of the germ layers, the follicular epithelium of the body cavity will take part in all these changes, and will thus conform to the shape of all the organs which are formed before it degenerates. Thus all the organs of the embryo will become outlined in both blastomeres and follicle cells, so that the follicle will become a sort of mold or cast of the embryo. These changes are so hard to draw and so easy to imagine that diagrams of them seem uncalled for.

The most remarkable peculiarities of the Salpa embryo are now to be described. They are due to another set of secondary changes which are quite anomalous.

After the follicle had become developed in the way which I have sketched, so that it formed a cast of the embryo, segmentation and the formation of the germ layers became retarded gradually, step by step, as compared with the development of the follicular layers, until the salpa embryo as we now know it was evolved. Cut L is a diagram of a salpa gastrula, and the character of its secondary modifications will be understood by comparing it with cut K.

The segmentation of the salpa egg is so much retarded that, in the gastrula stage, the germ layers are represented only by the scattered blastomeres, *3* and *9*. There is no digestive cavity, as the space between the scattered endodermal blastomeres, *9*, is completely filled with migratory follicle cells, as is the blastopore also; while the ectoderm is represented by a few scattered ectodermal blastomeres, *3*, external to the follicle in the region of the blastopore.

While the development of the true germ layers is thus retarded, the follicle cells follow, in their migration, the paths which were established for them before the retardation of the germ layers was initiated, and they now shape themselves into the cast of a gastrula, and this pseudo gastrula becomes converted into the simulacrum of a tunicate embryo, carrying with it the retarded blastomeres, in the way which has already been fully illustrated and described. The blastomeres thus reach their final positions in the body of the embryo before they become differentiated and arrange themselves in organs and tissues. Finally, the follicular layers break up. Many of their cells are taken into the substance of the blastomeres and digested, while others are left in the body cavity as free wandering cells, destined to degenerate at later stages and to serve as food for the tissues of the body.

In this section I have not been able to avoid the language of phylogeny, and I therefore wish to say once more that the account which is here given is both hypothetical and diagrammatic. The actual salpa embryo is complicated by the possession of a placenta, and by peculiarities which may be due to the former presence of a food-yolk which has disappeared. These features have been left out of consideration, and, even with this qualification, I have no desire to assert that, during the actual history of the evolution of the salpa embryo, it has moved along the path which I have indicated.

We have no history of its origin, and it is quite possible that the course of development has been very different from this imaginary reconstruction.

I believe, however, that the comparison throws light upon the fundamental nature of the embryo, and upon its relation to the embryos of other tunicates, and I claim that, so far, it is valuable, although I should be the last to assert its definite phylogenetic significance.

I have spoken of the history of the blastomeres as retarded, but it is probable that the retardation is not actual but only relative, and that the embryo is actually produced more quickly than it was before the follicular migration became established.

CHAPTER IV.

THE ORIGIN OF THE PROLIFEROUS STOLON.

My own observations on the origin of the stolon of Salpa are by no means in complete harmony with the accounts which have been published, but I shall adhere to the plan which I have followed so far, and shall try to describe my own observations briefly and simply, leaving literary comment for a later chapter.

Section 1.—*Outline Sketch.*

The aggregated salpæ are produced from a proliferous stolon, which grows out from the body of the solitary salpa. It makes its appearance while the solitary salpa is an embryo, and as it begins to become converted into chain-salpæ before this is born, its early stages must of course be studied in the embryo.

The stolon is a tube which is joined, at its proximal end, to the body of the solitary salpa, while distally it ends blindly. Its structure and its anatomical relations are well shown in the following figures. Plate XXXV shows at *a′* a longitudinal section of a very young stolon, and its position is intelligible, since the entire figure is a longitudinal section through the body of an embryo. This stolon is shown more magnified in Plate XX, Fig. 7, and a series of transverse sections through another stolon at the same stage is shown in Plate XX, Figs. 1, 2, 3 and 4. Plate XLI, Fig. 3, is an embryo a little older than the one in Plate XXXV, and its stolon is seen at *st*. In Fig. 4 of the same plate the same stolon is shown more enlarged, and it is shown in longitudinal section in Plate XVI, Fig. 5.

The stolon, *st*, of the embryo, in Fig. 5 of Plate XLI, is shown more enlarged in Fig. 6, and a series of transverse sections of the same stolon is shown in Plate XXI, Figs. 1–7. In this series, Fig. 1 is the most proximal section, at the root of the stolon, and Fig. 7 the most distal one, near its tip. A transverse section of an older stolon is shown in Plate XVI, Fig. 4; another, of a still older one, in Plate XLV, Fig. 5, and a section through the root or proximal end of the stolon of a fully grown solitary Salpa pinnata is shown in Plate XXXIV, Fig. 1.

As this series of figures shows, the stolon of Salpa pinnata lies below the middle line of the ventral surface of the solitary salpa, with its free end pointing forward. In several of the figures, as in Plate XVI, Fig. 4, Plate XXI, and Plate XLV, Fig. 5, the two folds d–d which compose the endostyle of the solitary salpa are shown, and it will be seen that the stolon lies under the furrow in the middle line of the ventral surface of the pharynx between these folds.

The figures in Plate XXI also show that the vertical longitudinal plane, between the halves of the endostyle, which cuts the solitary salpa into symmetrical halves, also cuts the stolon symmetrically, so that Plate XXXV cuts both the embryonic solitary salpa and the stolon in the plane of bilateral symmetry. When in its true or morphological position, then, the stolon is symmetrical with reference to the middle plane of the solitary salpa, although, as the stolon grows older, it is usually somewhat twisted on its own axis, as Plate XVI, Fig. 4, and Plate XLV, Fig. 5, show.

In many species of Salpa, the stolon, as it grows, becomes wrapped in a spiral around the digestive organs, as is shown at st, in the figure of the solitary Salpa africana, in Plate IV, Fig. 2, and when this is the case, the stolon begins to curve to one side almost as soon as it makes its appearance. There can be no doubt, however, that in all cases it is, morphologically, a median structure, symmetrical in the same plane as the solitary salpa; that Salpa pinnata shows its true or primary position, and that its position in species like Salpa africana is secondary.

When fully formed it consists, in cross section, Plate XXXIV, Fig. 1, of the following structures: 1st, a tubular sheath of ectoderm, a; 2d, a nerve tube, l; 3d, an endodermal tube, d'; 4th, two perithoracic tubes, g and h; 5th, two blood-tubes, i and j, and 6th, a genital rod, m–n. In addition to these well-defined constituents, there are isolated cells between the cloacal tube and the adjacent structures, shown, uncolored, in Plate XXXIV, Fig. 3.

Before the chain-salpæ make their appearance, all these structures run from the base of the stolon nearly to the tip, so that every section is like the figure, but at the tip the nerve tube, genital rod and perithoracic tubes disappear, and the endodermal tube d' ends blindly, as the longitudinal section, in Plate XVI, Fig. 4, shows, so that the two blood-tubes communicate with each other. They do not communicate with the exterior, however, or with the cavity of the endodermal tube.

SECTION 2.—*The Orientation of the Stolon.*

As the regions of the stolon undergo very great changes of position during its conversion into a series of salpæ, it is necessary to fix upon definite terms for its various regions. I shall call the tip of the stolon the *distal end.* Morphologically this end is anterior, and the end where it joins the body of the solitary salpa is posterior; but as in many species the stolon is not straight, but coiled, the terms distal and proximal are better, and I shall call the root of the stolon its *proximal end.* As the region along which the nerve tube lies is above, or towards the body of the solitary salpa, I shall call it the *top of the stolon,* and the opposite surface, where the genital rod lies, I shall call the *bottom.* The side which is on the right, when the solitary salpa is pictured as placed in front of the reader with its oral end above and its dorsal surface towards the reader, I shall call the *right side of the stolon.*

SECTION 3.—*The Ectoderm of the Stolon.*

This, like the ectoderm of the solitary salpæ and chain-salpæ, is colored purple in all the sections.

As Plate XVI, Fig. 5, and other figures show, it is continuous at the proximal end of the stolon with the ectoderm of the solitary salpa.

The multiplication of the ectoderm cells is the most efficient agent in the production of the tubular stolon. In Plate XLI, Fig. 2, x marks the place where the stolon is to be developed, although there is as yet no trace of it. Plate XX, Fig. 6, is a median longitudinal section through this region of the body of an embryo a little older, and the youngest in which I have found traces of the stolon. Plate XXXV will serve to show what part of the body is included in this figure, although it is from an embryo younger than Plate XXXV. In Plate XX, Fig. 6, f, colored yellow, is the pericardium; k is the eleoblast; n is the germinal mass, or aggregation of embryonic germ cells, in the body cavity of the solitary salpa embryo; a is the unmodified ectoderm of the embryo, and a' the tract of ectoderm which is to become the ectodermal tube of the stolon. The ordinary ectoderm cells of this region of the body are flat, but those which form this tract are cylindrical, and while none in active multiplication are shown in this figure, other sections show that they do multiply, and the ectodermal area of the stolon thus extends farther and farther backwards, as shown at a' in Figs. 5 and 7, until the germinal mass, Fig. 7, n, and the endodermal tube, d', are shut in, or covered by a

dome of ectoderm, as is shown at a' in Figs. 1, 2, 3 and 4, which are a series of sections through a stolon a very little older than Fig. 7, parallel to a line drawn from the letter c to the letter n. A stolon a little older is shown in surface view in Plate XLI, Fig. 4, and in longitudinal section in Plate XVI, Fig. 5. The dome of specialized ectoderm has here become a deep bowl, and its growing edge pushes farther and farther inwards, so as to form a double fold of ectoderm around the proximal end of the stolon. In Salpa pinnata this fold soon results in the formation, around the base of the stolon, of a prepuce-like structure, shown in Plate XLI, Fig. 6, so that, while the distal end of the stolon is covered by only one layer of ectoderm, its proximal end has, as Plate XLV, Fig. 5 shows, three layers: first, the ectoderm, A, of the solitary salpa; second, the reflected layer, A'', and third, the ectoderm, A', of the stolon.

The portion of the stolon which is covered by these three layers of ectoderm is often so much compressed that the structures included in it are crowded together, as Plate XVI, Fig. 4, shows, so that the space between the endodermal tube and the ectoderm appears at first sight to be filled up with a mass of undifferentiated cells, although more careful examination shows that all the structures which are shown in Plate XXI, Fig. 7, are really present. The effect of this compression will be understood by comparing this last figure with Fig. 4 of Plate XVI. The presence of three layers of ectoderm around the proximal end of the growing stolon gives great complexity to sections like those in Plate XXI. In these figures, a is the unmodified ectoderm of the solitary salpa, a' that of the stolon, and a'' the reflected layer, and the relations of the three layers are well shown in Fig. 6, which will serve to interpret the other figures.

I do not know whether these three prepuce-like layers of ectoderm are found in other species than Salpa pinnata. So far as I am aware they have never been described. Seeliger gives (6), Taf. XII, Fig. 7, a section of a young stolon of Salpa democratica at a stage in which he says that the space between the endodermal tube and the ectoderm is filled up by a solid mass of undifferentiated mesoderm. There is certainly no such stage in Salpa pinnata, and it is possible that his figure represents a compressed section like my Fig. 4 in Plate XVI. I shall, however, discuss his view of the origin of the stolon further on.

SECTION 4.—*The Nerve Tube.*

This is colored violet in the sections, like the ectoderm, as it is ecto-dermal in origin, although very young stolons must be studied to show this. In all the older stolons it is distinct from the ectoderm from end to end, and appears in all the sections, as it is shown in Plate XXXIV, Fig. 1, at *l*, as a closed tube, with a lumen, in contact with the inner surface of the ectoderm, on the middle line of the upper surface of the stolon. Even in a stolon as young as the one shown in Plate XXI, sections throw no light on its origin, for Fig. 7 shows that it has the structure that it has in the figure just referred to. In the stolon, a little older than Plate XX, Fig. 7, which is shown in transverse section in Figs. 1, 2, 3 and 4, I found the clearest evidence of its ectodermal origin. In the more proximal sections 1, 2 and 3, the ectoderm covers only the bottom and sides of the stolon, but the tip of the distal end, Fig. 4, is completely shut in, although its ectoderm, *a'*, hås not yet separated from the ectoderm, *a*, of the solitary embryo, and at the point where the two layers are continuous, the abundance of nuclear figures shows active cell multiplication, and a number of the cells have pushed in to the cavity of the stolon, where they form a projecting knob, *l*, the rudimentary nerve tube. At this early stage it is solid, and its lumen, which appears later, never has any communication with the exterior. At least I have found no opening, although Salensky, who has recently shown (17) that the nerve tube of the ascidiozooid of Pyrosoma is ectodermal, finds a dis-tinct neural invagination. A longitudinal section of the somewhat older stolon of Plate XLI, Fig. 3, is shown in Plate XVI, Fig. 5. Here the nerve-rudiment, *l*, stretches for some distance along the middle line of the stolon, and at its distal end the cells are arranged around its axis, although the lumen has not yet made its appearance. Plate XXXV shows that the stolon is about as far away from the ganglion of the solitary embryo as it could well be, and it is difficult to believe that there is any phylogenetic connection between the nerve tube of the stolon and the ganglion of the embryo. It is of course possible that ontogenetically the two structures may be common descendants of the same cells, but there is no indication of any such ontogenetic relation-ship. Uljanin believes (7) that the budding of salpa is to be traced back, phylogenetically, to fission in a double embryo. If this has been its history we certainly should expect to find corresponding structures arising in positions which are more consistent with their supposed

fundamental identity of origin. I shall, however, discuss the subject further on.

SECTION 5.—*The Endodermal Tube.*

This is colored red in the sections, as are also its derivatives.

As d' in Figs. 2, 3, 4 and 5 of Plate XIX shows, it arises as a tubular outgrowth from the ventral wall of the pharynx on the middle line between the two folds of the endostyle, which are marked d–d in these and other figures; and in Salpa pinnata and Salpa cylindrica it permanently retains its communication with the cavity of the pharynx. Seeliger states that in Salpa democratica it soon loses its communication with the pharynx, but this species is a difficult one to study, and the opening is easy to find in the straight stolons of the two species which I have studied most thoroughly, even in the oldest specimens. I have not found in any of my sections any food or foreign organisms in the endodermal tube of the stolon at any stage, nor have I ever found in the stomachs of the young chain-salpæ any evidence that they are nourished through it, although their digestive organs are derived from it, and retain for a long time their communication, through it, with the pharynx of the solitary salpa. It is formed, as Plate XXXV shows, before the mouth of the solitary embryo communicates with the exterior, and there is no reason for believing that it has a nutritive function. As the longitudinal sections in Figs. 5, 6 and 7 of Plate XX show, the inner ends of its cells have a ragged or indefinite outline, as have also the cells of the endostyle, Fig. 2, d, and it is probably ciliated when alive, and, at later stages, when the pharynx of the solitary salpa is filled with fresh sea-water, it may, perhaps, convey to the young chain-salpæ a respiratory current.

In a mature stolon, Plate XXXIV, Fig. 1, d', its upper and lower walls are formed of flattened cells, with clearly defined outlines, shown more magnified in Fig. 3, while along each side of it there is a thick strip of elongated cells, with their inner edges indefinite and ragged. At the distal end of the tube these two strips unite, and as the longitudinal section in Plate XVI, Fig. 5, shows, its tip consists of elongated cells.

Before we examine its structure and origin in detail, it will be necessary to briefly describe the endostyle of the solitary embryo. Plate XXI, Fig. 5, shows in red at b–d–d–b a transverse section across the middle line of the pharynx of the solitary embryo. The ordinary endoderm of the pharynx is shown at b. It consists of flattened cells with sharp outlines. The endostyle consists of two parallel ridges of greatly elongated cells

with indefinite outlines, separated from each other on the middle line by a deep furrow, the floor of which consists of flat, sharply defined cells, like those of the pharynx in general.

At the stage shown in Plate XX, Fig. 7, where the ectoderm of the stolon forms a shallow bowl or dome, the endoderm grows down into the cavity of the bowl, as shown in the transverse sections in Figs. 1, 2 and 3, to form the endodermal tube, which is at first widely open above into the cavity, c, of the pharynx, and consists of a floor of flattened, sharply defined cells, which is part of the floor of the endostylic furrow of the solitary embryo, and which crosses the middle line of the stolon to join at each end a vertical wall of thickened cells, with indistinct and irregular inner ends, like the cells in the endostylic ridges. As Fig. 1 shows, these side walls of the endodermal tube are actually part of the endostylic ridges, into which they can be traced in Fig. 1. Soon, however, as the older or more distal section in Fig. 2 shows, the endostylic ridge proper becomes separated from the endostylic side wall of the stolon by an intervening area at d–d in Fig. 2, where the cells become flat and sharply defined. In a still more distal or older section, Fig. 3, these two belts of flattened cells are pushed towards each other until they meet on the middle line to form the roof of the endodermal tube. The way in which this tube is formed shows that it is actually a section of the endostyle which drops down out of the solitary embryo into the stolon.

As the solitary embryo develops, its two endostylic ridges come nearer to each other, and finally meet on the middle line, as the successive stages, in Plate XXI, Plate XVI, Fig. 4 and Plate XLV, Fig. 5 show, and the channel which connects the cavity of the endodermal tube with that of the pharynx of the solitary salpa is thus reduced in size until it becomes a small slit, d' of Plate XXI, Fig. 1.

Within the stolon the endodermal tube assumes the shape shown in the figures in Plate XXI. In cross-section it is a little like a letter H, and is made up of a horizontal cross-bar and two vertical ends. In young stolons the floor and roof meet and come into contact with each other on the middle line, so that the cavity is divided into two lateral chambers which communicate with each other only at the distal end, Plate XX, Fig. 4, but as the stolon grows older the roof and the floor separate from each other, as Plate XLV, Fig. 5 shows, until in mature stolons the cavity assumes the shape which is shown in Plate XXXIV, Fig. 1, d'.

SECTION 6.—*The Blood-Tubes.*

There are two of these, the upper one, j, and the lower one, i, both colored yellow in the sections. They communicate with each other at the tip of the stolon, around the tip of the endodermal tube. Elsewhere they are separated from each other by the endodermal tube and the perithoracic tubes, which are so placed as to form a horizontal longitudinal partition. In their origin the tubes are part of the cavity of the body of the solitary embryo, and they are present in the stolon at all stages. When the ectoderm and endoderm which are to enter into the structure of the stolon first become differentiated from the tissues of the embryo, Plate XX, Fig. 6, these two layers are in contact with each other, and, as Plate XLI, Fig. 7, shows, both the ectoderm, a, and the endoderm, b, are in direct contact with the germinal mass, n, but as soon as the rudimentary stolon begins to elongate, Plate XX, Fig. 7, the endoderm separates a little from the other structures, so that there is space for the blood corpuscles of the solitary salpa to circulate between them, and these spaces are present at all subsequent stages, as the transverse sections in Plates XX and XXI, and the longitudinal section in Plate XVI, Fig. 5, show.

The lower blood space, i, is the first one to be folded into the stolon, as shown in Plate XXI, Figs. 1 and 2, i; and in Plate XXXV it is continuous with that part of the body of the solitary salpa which contains the eleoblast, k. I shall show farther on that the degenerating cells of the eleoblast furnish food for the blood corpuscles and migratory mesoderm cells of the embryo, and as the sections of the stolon show that these latter pass into it in great numbers, they no doubt play an important part in the nutrition of the growing stolon and the chain-salpæ which are formed from it. The upper blood space, j, is in intimate relation with the heart, e, and the great ventral blood-vessel of the solitary embryo, and it will be necessary to say a few words here about these structures. The pericardium, f of the sections, and the heart, e, are shown in horizontal sections of the solitary embryo, in Plate XIX, and in vertical section in Plate XXXV. The pericardium is a closed vesicle, colored yellow in the figures, behind the pharynx, c, and to the left of the middle line, and the heart is formed by the infolding of its walls on the side next the pharynx, as shown at e in Plate XIX, Figs. 1, 2, 3, 4 and 5. In its origin the chamber of the heart is thus part of the body cavity, external to the pericardium. In the young embryo it is behind the pharynx, c, and

vertical. It is at first simply a furrow, and its upper and lower ends remain permanently open, but in its middle region, Fig. 3, more magnified in Fig. 9, its lips fold in and meet so as to convert it into a tube. Its lower opening is shown at *e* in Fig. 5, and more enlarged in Fig. 10, and its upper opening at *e* in Fig. 1, more enlarged in Fig. 6. The upper end of the heart, Plate XXXV, is situated at the base of the gill, *o*, which is a tubular rod bathed on all sides by the water which circulates through the pharynx, *c*, and cloaca, *g'''*. In the mature salpa the lower end of the heart communicates with a large blood space or vessel which runs along the middle line of the ventral surface under the endostyle. This vessel is shown in section, not marked by a letter, in Plate XXI, Fig. 7, and also in Plate XVI, Fig. 4. It is also shown in a young chain-salpa, in Plate XXXVIII, Fig. 29, at *j*. In the young solitary embryo it is very short, and it opens into that part of the body cavity which lies over and around the placenta.

Plate XXI, Fig. 1, cuts the heart, *e*, and the pericardium, *f*, of the solitary embryo. Fig. 2 shows on the side next the stolon the epithelium of the pericardium and that of the heart in contact with each other. Figs. 3, 4, 5 and 6 pass through the inferior opening of the heart, and Fig. 7 cuts the ventral blood-vessel.

If the upper blood space of the stolon, *j*, of Fig. 7 be traced back through sections 7, 6, 5, 4 and 3, it will be seen that it communicates with the opening of the heart of the embryo, and through the ventral blood-vessel, with the placental portion of its body cavity, and it is therefore clear that it stands in very intimate relation to the two sources of nutrition, the degenerating eleoblast and the placenta, as well as to the gill.

When the heart of the chain-salpa is beating in one direction, blood flows from the eleoblast into the lower tube of the stolon out to its tip, and back to the heart through the upper tube. In the reversed circulation, blood from the gill of the embryo is driven by its heart into the upper tube, and out to the tip of the stolon and back to the eleoblast.

In the mature stolon, Plate XXXIV, Fig. 1, each blood tube has its own endothelial lining, which lies in such close contact with the other organs of the stolon that it easily escapes observation at all points except the places where it bridges over the gap between the ectoderm and the endodermal tube, Fig. 3, or in the angles at the sides of the nerve tube. It is shown in Plate XXIII, Fig. 10, *j*, and in Plates XXIV and XXX.

It is difficult to determine whether this endothelium is formed in place, as is probably the case, or derived from the endothelium of the blood spaces of the embryo, for its great delicacy in young stolons renders it unfavorable for study in sections. A fragment of it is shown in Plate XXI, Fig. 6, above the letter g, and it is also shown in Figs. 2, 3, 4 and 5.

SECTION 7.—*The Perithoracic Tubes.*

These are colored green in the plates; and the right one is marked g, and the left one h.

They run along each side of the stolon, between the ectoderm and the thickened side walls of the endodermal tube. In mature stolons, each of them has a distinct tubular lumen, as is shown in transverse section in Plate XXXIV, Fig. 3, h, and in longitudinal section in Fig. 6, h. In very young stolons, Plate XXI, Fig. 7, g and h, the lumen is absent.

I have devoted especial attention to the question of the origin of these tubes, but I have not been able to obtain any evidence which I regard as conclusive, although my observations indicate that the tubes arise in the ectoderm of the young stolon, at the points where this joins, at the sides of the stolon, the ordinary ectoderm of the embryo. In old stolons, like the one shown in Plate XXXIV, Fig. 1, the tubes can be traced to the proximal end of the stolon, and at the point where the ectoderm bends outwards to join that of the solitary salpa they come to an end, although I have not been able to find, in old stolons, any union between them and the ectoderm. The stolon shown in the transverse sections in Plate XX had no perithoracic tubes, although, on the left side of Fig. 3, the fold where the ectoderm, a', of the stolon joins that of the embryo, a, runs down for some distance into the stolon, alongside the endoderm.

This may possibly be the rudiment of a perithoracic tube,—the right one, as these sections were drawn from inverted specimens,—but I have not been able to prove to my own satisfaction that it is.

In Plate XXI, Fig. 2, the left perithoracic tube is shown to lie in very intimate relation to the ectoderm at the root of the stolon close to the point where, as section 1 shows, the ectoderm folds upon itself. This section, like the one before it, seems to show that the perithoracic tubes arise from the ectodermal fold at the proximal end of the very young stolon, and I know of no theoretical ground for doubting this evidence, although none of my sections show any vegetative activity in the cells

at this point.   A horizontal longitudinal section through a very young stolon would probably furnish more decisive evidence, but all my own specimens of the proper stage were used for other purposes.´

SECTION 8.—*The Mesoderm of the Stolon.*

In addition to the blood corpuscle and the cells which form the walls of the blood spaces, a number of cells become shut into the stolon on each side, between the ectoderm and the perithoracic tube, as shown in Plate XXI, and in Plate XXXIV, Fig. 3, while others are found, as Plate XXXIV, Fig. 3 shows, between the endoderm and the perithoracic tube. Some of these cells appear to enter the stolon as detached mesoderm cells, while it is possible that others are derived from the pericardium, for this unquestionably enters into the structure of the stolon.   On the left side of the inverted section in Plate XX, Fig. 1, it will be seen that a diverticulum from the pericardium, *f*, pushes in between the ectoderm and the right side of the endodermal tube, and Figs. 4 and 5 of Plate XXI show that the end of this process is constricted by the growing ectoderm, and a part of it, at least, probably becomes included in the stolon, although at an older stage there is nothing which can be identified as its derivative, and if it is represented at all it is probably represented only by independent cells.

Another constituent of the stolon may be mentioned here, the gelatinous substance which fills the spaces in the angles between the endothelium of the blood spaces and the other organs.   This substance, which is homogeneous and transparent, is shown at *32* in Plate XXXIV, Figs. 3 and 5.

SECTION 9.—*The Genital Rod.*

The origin and history of the genital rod will be treated at length in another chapter.

We must note here, however, that its rudiment, Plate XLI, Fig. 7, is present in the body cavity of the embryo before the stolon is formed, and that it is shut into the stolon, as the figures on Plate XX show, by the growth of the ectoderm.

SECTION 10.—*The Derivatives from the Parts of the Stolon.*

The ectoderm of the stolon gives rise to the ectoderm of the chainsalpæ, to the organs by which they are fastened to each other after birth,

and to their cellulose mantles. The nerve tube gives rise to the ganglia. The lateral portions of the endodermal tubes give rise to the corresponding halves of the pharynx; and the œsophagus, stomach and intestine are derived from the one on the right side. The perithoracic tubes give rise to the cloaca and to the cloacal portions of the two gill-slits by which it opens into the pharynx.

The body cavity consists, in part at least, of diverticula from the blood spaces, and it is lined by their endothelium. The muscles and the stoloblast, or the equivalent of the eleoblast, are derived from mesoderm cells from both sides of the stolon. The heart and pericardium are probably formed from some of the mesoderm cells on the right side. The eggs and their follicles and fertilizing ducts, and the testes, are derived from the genital rod.

CHAPTER V.

THE TRANSFORMATION OF THE STOLON INTO THE SERIES OF AGGREGATED SALPÆ.

SECTION 1.—*Outline Sketch.*

The origin of the aggregated salpæ is complicated by secondary changes, but in its essential features it is a very simple history. I shall therefore preface my account by a brief outline of the process, stated in its simplest form, and divested of all secondary complications.

I. *The Proliferous Stolon.*—As already described, this, when fully formed, consists, 1st, of a tubular sheath of ectoderm, Plate XXXIV, Fig. 1, a, which is derived from the ectoderm of the solitary salpa; and which contains, 2d, an endodermal tube, d', which is derived, as Plate XXI, Fig. 1, d' shows, from the pharynx of the solitary salpa; 3d, a nerve tube, Plate XXXIV, Fig. 1, l, which is derived, as Plate XX, Fig. 4, l, shows, from the ectoderm of the stolon; 4th, of two perithoracic tubes, g and h, which are probably derived from the ectoderm of the stolon, as shown in Plate XX, Fig. 3; 5th, an upper hæmal tube, j', which communicates at the base of the stolon with the cavity of the heart of the solitary salpa, as is shown at j in Plate XXI, Fig. 3; 6th, a lower hæmal tube, i, which communicates with the body cavity of the solitary salpa, as is shown in Plate XXI, Fig. 2, i; and 7th, of a genital string which consists of a series of eggs, m, inclosed in a follicular sheath, n, and which is derived from the germinal mass, Plate XX, Fig. 6, n, of the solitary salpa.

II. *The Segmentation of the Stolon.*—The first indication of the segmentation of the stolon is a series of ectodermal folds, Plate XXXIV, Fig. 11, a, which first appear at its sides, but soon extend up and down and completely encircle it, and, pushing inwards, mark out the body cavities of the salpæ, and also cut up the tubular structures inside the stolon into segments.

The active agent in this process of segmentation is the growth of the ectodermal folds, and the other structures are actually cut by these folds,

as Plate XXXIV, Fig. 10 and Plate XXIII, Fig. 7 show. As the result of this process the nerve tube becomes cut up into a series of ganglia, one for each salpa, Plate XV, Fig. 10, *s*; the perithoracic tubes become cut up into a series of perithoracic vesicles, two for each salpa, Plate XXXIV, Figs. 9, 10 and 11, and Plate XV, Figs. 11 and 12, *g* and *h*; the genital string becomes cut up into a series of eggs, Plate XV, Fig. 2, *n*, one for each salpa, inclosed in a follicle, *m*; and the thickened endodermal epithelium at the sides of the endodermal tube, *d'*, becomes cut up into a series of vertical pouches or pockets, two for each salpa, the rudiments of the right half of the pharynx, *27*, and of its left half, *28*, as is shown in Plate XXIII, Figs. 4, 7, 8 and 9, and Plate XV, Figs. 6, 7, 8, 11 and 12, where these pouches are colored red.

III. *The Rudimentary Chain-Salpa.*—The structures which I have enumerated form the rudiments of a single salpa. They are shown in Plate V, Fig. 1, which is a reconstruction from a series of tranverse sections through the stolon of Salpa pinnata.

At this stage each salpa is bilaterally symmetrical, and its plane of symmetry is the same as that of the stolon, while its long axis is at right angles to that of the stolon, which becomes converted into a single row of salpæ, so placed that, as is shown in cut M, the dorsal surfaces of all of

CUT **M.**
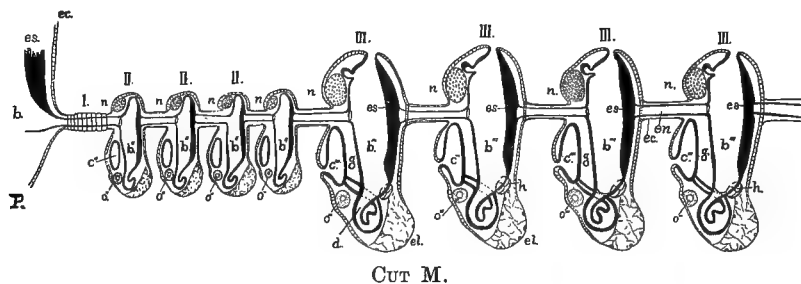
them are towards the base of the stolon, their ventral surfaces towards its tip, their right and left sides on its right and left respectively, their oral ends at its top or neural side, and their aboral ends at its bottom or genital side.

IV. *The Secondary Changes.*—The single row of salpæ becomes converted into a double row, Plate IV, Fig. 1, which consists of a series of right-handed salpæ and a series of left-handed ones, placed with their dorsal surfaces out, their ventral surfaces towards the ventral surfaces of those in the opposite row, and the left sides of those on the right and the right sides of those on the left towards the base of the stolon. In

order to illustrate these secondary changes of position let us represent the series of salpæ by a file of soldiers, all facing the same way. Now imagine that each alternate soldier moves to the right, and the others to the left, to form two files still facing the same way. Now let them face about so that the backs of those in one row are turned towards the backs of those in the other row. They will now represent two rows of salpæ like those shown in Plate IV, Fig. 1.

To make the illustration more perfect suppose that, instead of stepping into their new places the soldiers grow until they are pushed out by mutual pressure, and suppose that their heads, growing fastest, form two rows while their feet still form one row, and suppose furthermore that as each soldier rotates his feet turn first, and that the twist runs slowly up his body to his head, which turns last. We must also imagine that these various changes all go on together, and that while they are taking place each soldier not only grows larger, but also develops from a simple germ to his complete structure.

As thus outlined these secondary changes are simple, but as growth, development, pushing to the side, and rotation are all going on together, and as they all take place gradually, the interpretation of sections is very puzzling; although the history of their development becomes very simple when it is illustrated by diagrams from which the secondary changes are omitted, instead of by figures from actual sections.

V. *The Development of the Chain-Salpa.*

(1) *The body cavity.* At first this is directly continuous, through the blood spaces *i* and *j*, with those of the adjacent salpæ, but it is gradually shut in by the growth of the ectodermal folds, of which the inner edges is shown in the diagrams and in the figures on Plate V.

(2) *The pharynx.* The lateral pouches, Plate V, Fig. 1, *27* and *28*, from the sides of the endodermal tube, *d'*, grow forwards towards the oral end of the body, cut N, *27* and *28*, *o*, and backwards towards the aboral end, cut O, *27* and *28*. They are the rudiments of the right and left halves of the branchial sac or pharynx, and they may therefore be called the *pharyngeal pouches.*

Their oral ends soon bend in towards each other, cut O, and finally meet and unite on the middle line, cut P, ventral to the ganglion, *s*, to form the oral end of the pharynx, which is shown at *28–o–27* in cuts P to X. Finally, the oral end of the pharynx unites with the ectoderm and becomes perforated to form the mouth, cut S, *z*.

CUT N.

CUT O.

CUT P.
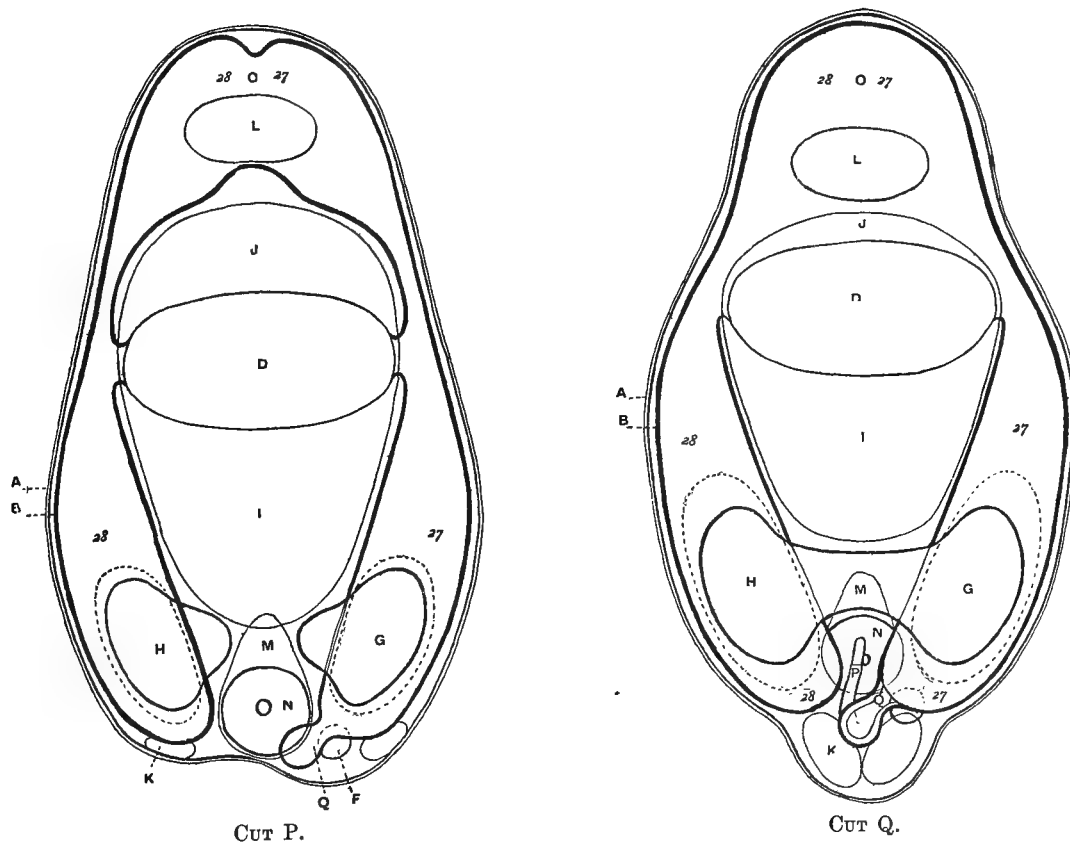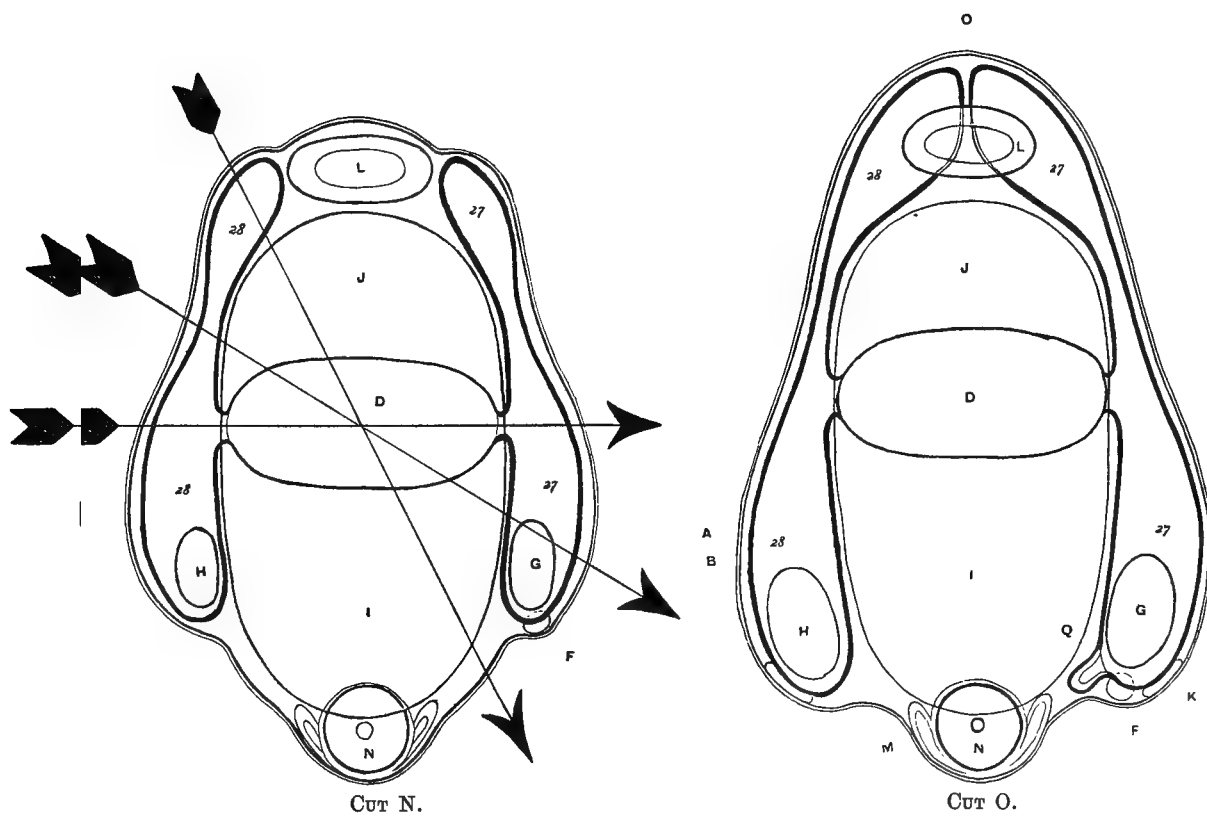
CUT Q.

The aboral ends of the pharyngeal pouches elongate, as is shown in cuts O and P, *27* and *28*, and, bending towards each other, cut Q, finally meet on the middle line and unite with each other, as shown in cut R, *27–28*, to form the aboral portion of the pharynx. They are shown in contact but not yet united, in Plate XXXIII, *L–L'*, Figs. 3, 4, 5, *27* and *28*, while in *M–M'* and *N–N'*, Figs. 3 and 4, they are united on the middle line of the ventral surface.

Both the oral and the aboral ends of the pouches ultimately unite on the middle line throughout their whole length, so that, as is shown in cut S and cut X, the pharynx becomes a large, unobstructed chamber,

CUT R.   CUT S.
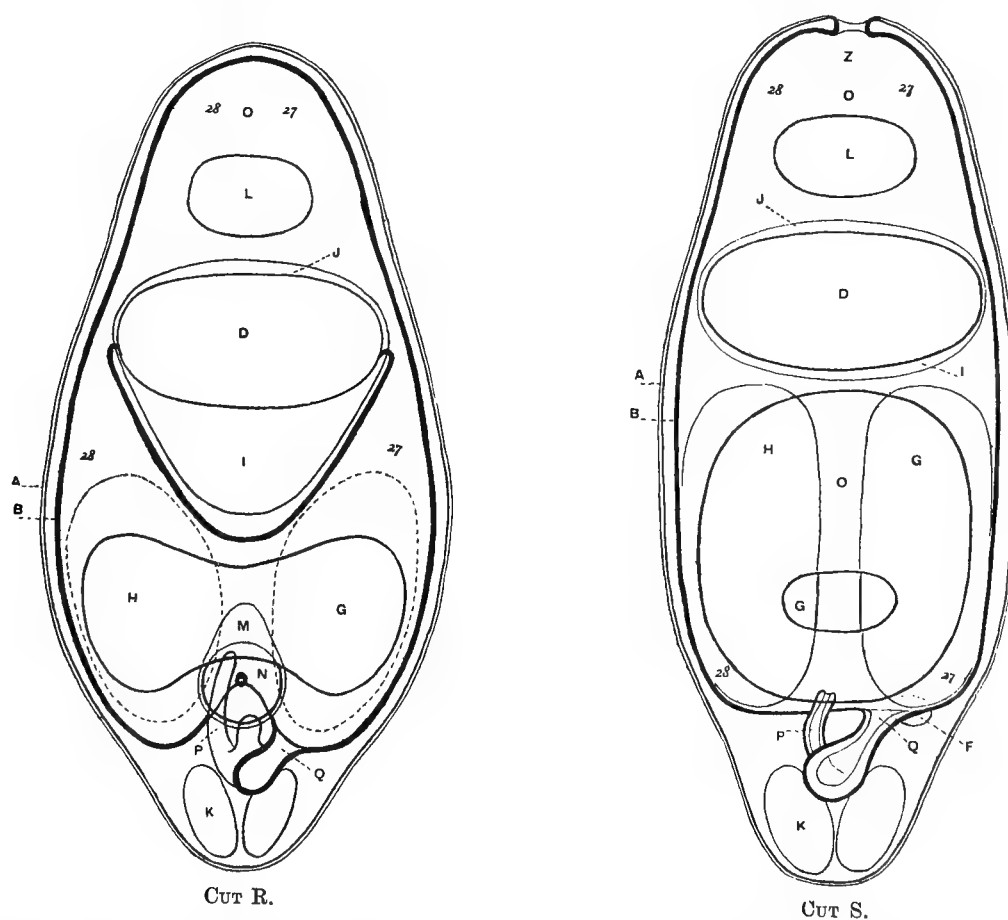
opening to the exterior at the mouth, and communicating with those of adjacent salpæ through the opening which is marked *d'* in the figures.

(3) *The perithoracic tubes and cloaca.* As the pharyngeal pouches grow backwards they carry with them, on their dorsal sides, the perithoracic vesicles, *g* and *h*, as is shown in cut N. At about the stage shown in cut O, each of these vesicles unites with the dorsal surface of the

pharyngeal pouch and opens into it, as is shown in section in Plate XXVI, Fig. 3, *E–E'*, where the perithoracic vesicles, *g* and *h*, colored green, are cut through their openings into the pharyngeal pouches, *27* and *28*, which are colored red. These two openings are the two gill-slits, and in cuts P, Q and R their edges are indicated by dotted lines. Comparison of the figures will show that they soon undergo a very great increase in size, until at the stage of cut S they include all the dorsal surface of the aboral end of the pharynx except a strip, *o*, on the middle line.

Each perithoracic vesicle, after it has established its communication with the pharyngeal tube, gives rise to a diverticulum, cut P, which grows inwards towards the middle line of the dorsal surface, as is shown in Plate XXV, Fig. 7, *H–H'*, *g* and *h*, and these diverticula ultimately meet and unite on the middle line, as is shown in cut Q, to form the cloaca, *g'''*. Plate XXXIII, Fig. 3, *K–K'* shows, colored green, these two diverticula meeting on the middle line of the dorsal surface, which is the lower surface in the sections.

Soon after the cloaca is thus formed its dorsal wall unites with the ectoderm on the middle line, and the cloacal aperture, *G*^{v} of cut S, is formed at the point where this union has taken place. Plate XXXVI, Fig. 5, *g*^{v} shows the union between the dorsal wall of the cloaca and the ectoderm, and it is shown again more advanced in Plate XXXVIII, Figs. 88 and 97, *g*^{v}, and also in Plate VIII, Figs. 1 and 2, *g*^{v}.

(4) *The gill.* The gill of salpa, cut S, *o*, and Plate XXXVI, Figs. 5 and 6, *o*, is simply that part of the body cavity which is bounded ventrally by the dorsal wall of the pharynx, dorsally by the ventral wall of the cloaca, and at the sides by the gill-slits.

(5) *The digestive organs.* The œsophagus, stomach and intestine are formed by gradual specialization in the course of the tubular diverticulum which is shown at *q* in cut P, growing out from the posterior end of the right pharyngeal pouch, *27*. In cut P, its blind end is dilated to form the stomach, and in cut Q, the intestine, *p*, is shown growing out from the ventral side of the stomach and bending upwards towards the dorsal surface, where in cut S it opens into the cloaca.

(6) *The pericardium and the heart.* The pericardium makes its appearance very early, cut O, as a closed vesicle, *F*, external to the aboral end of the right pharyngeal pouch, *27*. As this pouch lengthens it carries the pericardium back with it, cuts P and Q, *F*, and Plate XXIV, Fig. 2, *e*, and as the digestive tract is formed it pushes the heart towards the ventral surface, cut S, *F*, and Plate XXV, Fig. 4, *H*, *e*, and Fig. 7, *F*,

e, and it finally comes to lie under the ventral surface of the right side of the pharynx, Plate XXXVI, Figs. 3, 4 and 5, *f*. As shown in these figures, the heart arises as a furrow which is formed by the involution of the dorsal surface of the vesicular pericardium.

This outline of the history of the larger organs of the chain-salpa will give enough insight into the mode of development of its body to enable the reader to understand the more minute and detailed description which follows.

SECTION 2.—*The General Characteristics of Aggregated Salpæ.*

The way in which the proliferous stolon grows out from the body of the salpa embryo and gradually acquires its complicated organization may be spoken of with perfect propriety as *budding;* but inasmuch as the stolon itself contains the rudiments of all the important systems of organs, its transformation into a series of aggregated salpæ by cell multiplication and by the folding of its various parts, is more like the development of an embryo with germ layers into the body of a complicated adult than it is like ordinary budding, and it may perhaps be more proper to speak of it as the *strobilization* of the stolon. It is of course a process of asexual multiplication, but as the stolon contains the potency of all the following generations of chain-salpæ, it stands, as Seeliger has pointed out (11, p. 583), in somewhat the same relation to the chain-salpæ as that which a young embryo, with its future structure latent in its germ layers, bears to the perfect adult, since, in each case, the process of development consists in the unfolding of its rudimentary organization.

As a knowledge of the final result will aid us in tracing the minute details of the process, I shall give a short outline of the most conspicuous features before I describe the process of strobilization.

The growing stolon lies in a chamber which is hollowed out in the cellulose mantle of the solitary salpa, and as the aggregated salpæ are set free they escape through an opening which connects this chamber with the exterior.

In Salpa pinnata, Plate I, Fig. 6; Salpa chamissonis, Plate I, Fig. 7; Salpa cylindrica, Plate III, Fig. 7, and in a few other species, the stolon lies underneath the middle line of the ventral surface of the body of the solitary salpa, and it is symmetrically placed, with its free or distal end pointing forward, and with its right and left sides symmetrically placed with reference to the plane of symmetry of the solitary salpa.

In Salpa democratica, Plate II, and in most of the species of salpa, it becomes asymmetrical almost as soon as it is recognizable, and it grows around the nucleus in a spiral, as is well shown by Seeliger's excellent figure of the stolon of Salpa democratica (11, Taf. X, Fig. 5), and by his description (11, p. 593), and also in my figure of the solitary form of Salpa africana, Plate IV, Fig. 2, st.

In Salpa pinnata, Salpa chamissonis, and probably in all the pinnata-like species, the stolon presents a graduated series of stages of development; each successive salpa, from the root of the stolon to its tip, being a little larger and more developed than the one behind it, as is shown in Plate XLVI, and also in the series of figures on Plates XXIII to XXXIII. In these species the salpa at the tip of the stolon is largest and most developed, and new ones are continually being marked out at the base of the stolon as those at the tip are set free.

In all the other species the chain-salpæ are developed in sets, as is shown in the cut M, and all the individuals in a set are in essentially the same stage, although there is gradation among the members of the youngest set at the root of the stolon. The diagram does not show the number of individuals in each set. They are always very numerous, and in some species each set contains a hundred or more.

We know nothing of the birth-rate of salpa, but the solitary salpa begins to set free chain-salpæ soon after it is born. I have never found a specimen with an exhausted stolon, and there is no evidence of any fixed limit to the process of asexual multiplication. The number of buds on the stolon at one time is very great. Salpa democratica usually has three or four sets at one time, and Leuckart (1, p. 67) found forty in one set and sixty-five in another. Seeliger says (11, p. 593) that he counted sixty-one in a single set in this species, and the average is probably about sixty in each set, or between two hundred and two hundred and fifty in all on the stolon at one time. The stolon of Salpa pinnata has about the same number, from two hundred to two hundred and fifty. The number is very much greater in Salpa cylindrica, and I have counted two hundred in a single set from this species. As its stolon carries three or four sets, the total number of buds at one time is from six hundred to eight hundred.

The position which the bodies of the aggregated salpæ occupy when they are first marked out in the stolon has been much misunderstood. I shall show soon that, morphologically, they form only a single series, and that they all arise in exactly the same position, with the neural or dorsal

surfaces of all turned towards the root of the stolon, with all their right sides on the right of its plane of symmetry and their left sides on its left, and with their long axes at right angles to the long axis of the stolon, and their oral ends above, as they are shown in the diagram, cut M. As they increase in size, however, and become crowded, they push out of the line to the right and left alternately, and thus form two ranks instead of one. At the same time the body of each salpa rotates ninety degrees upon its own axis, so that the neural or dorsal surfaces come to face outwards, while the left sides of those on the right and the right sides of those on the left become turned towards the base of the stolon, and the planes of symmetry of the salpæ, instead of coinciding with the plane of symmetry of the stolon, make right angles with it. It is most important to grasp clearly the fact that this position is a secondary one, and that, morphologically, there is only a single series of animals, all placed in the same position and all facing the same way like a single file of soldiers; for the change of position takes place at a very early stage, and all the published accounts of the budding of salpa are so vitiated by a failure to discover it, or else to understand it, that they are almost worthless.

The chain-salpæ of Salpa scutigera, Plate IV, Fig. 1, retain this secondary position after they are born, but in most species this arrangement, which persists in Salpa scutigera, is transitory, and still other changes of position soon take place.

The position which is shown in cut M may be called *position one*, and the position which persists in Salpa scutigera, *position two*. In every species the salpæ arise in the stolon in position one, which may therefore be called the true, or *morphological*, position, and in every species they quickly assume position two. Plate VIII, Fig. 1, shows two of the aggregated salpæ of Salpa pinnata in this position, and Fig. 2 two of Salpa cylindrica.

This position is also shown in sections of Salpa pinnata in Plates XXXVI, XXXVII and XXXVIII, and in sections of Salpa cylindrica in Plates XXXIX and XL. These plates, and Plate XV and Plates XXIII to XXXIII, represent sections which are parallel to the long axis of the stolon, and transverse to the bodies of the salpæ, and in all of them the right side of the stolon is on the right side of the figure, and its proximal end towards the bottom of the page.

In Salpa pinnata, Plate I, Fig. 2, and Salpa chamissonis, Plate XLI, Fig. 9, only a few aggregated salpæ, about eight in Salpa pinnata and twelve in Salpa chamissonis, are set free at one time, and these, just before they escape, arrange themselves in a wheel or rosette with their

dorsal surfaces outwards, and their long axes parallel to the axis of the wheel. In all my preserved specimens the tip of the stolon had been so much flattened by contact with the side of the bottle, in transportation, that I have not been able to study in detail the way in which this wheel-like arrangement is acquired, and the subject should receive the attention of those who are able to study living specimens.

There is an obvious resemblance between the wheel-like arrangement of the first four ascidiozooids which in Pyrosoma form the basis for the cylindrical community, and the wheel-like aggregation of the Salpæ in species of the pinnata group, as may be seen by comparing Salensky's figures of the young Pyrosoma community (17, Taf. II) with Plate XLI, Fig. 9. In each case the animals are arranged in a circle with their long axes parallel to the central axis, their dorsal surfaces outward and their oral ends above. I shall show that there are other reasons for believing that the pinnata-like species of Salpa are most primitive and most closely related to Pyrosoma, and it is not improbable that the wheel-like arrangement has been inherited in both cases from a common source, and that it is the primitive arrangement for the Salpæ.

In most species, however, the aggregated salpæ are set free in the well-known floating clusters which have long been called chains. Part of a chain of Salpa cordiformis is shown in Plate IV, Fig. 6, and part of one of Salpa scutigera is shown in Plate IV, Fig. 1. As is shown in these figures, a chain consists of two parallel longitudinal rows of individuals, so placed that those in one row alternate with those in the other, while the neural or dorsal surfaces of all are external, and their ventral surfaces in contact with the ventral surfaces of those on the other side of the chain.

The members of the community are united to each other by process from the walls of their bodies, which are hollow and contain diverticula from their body cavities, although there is no communication between the body cavities of adjacent salpæ.

In the pinnata group, Salpa pinnata, Plate I, Fig. 3; Salpa chamissonis, Plate VIII, Fig. 6; Salpa affinis and Salpa dolichosoma, there is only one of these processes, situated on the middle line of the ventral surface in front of the heart. The way in which it arises is well shown in the figures in Plate VII, Figs. 4 and 5, and Plate VIII, Fig. 1. Plate I, Fig. 3, shows it in Salpa pinnata in its perfect form, while the other figures show it at earlier stages. It is also shown in section, at successive stages, in Plate XXXVI, Fig. 9, Plate XXXVII, Figs. 10 and 21, and Plate XXXVIII, Figs. 52 and 61.

These figures show that the process is ectodermal, hollow, and that the ectoderm of adjacent salpæ comes into contact at the end of the process. After the cellulose mantle is formed the processes come to consist almost entirely of cellulose, although the actual contact between adjacent salpæ is ectodermal. Plate XXXVII, Fig. 21, shows that in Salpa pinnata each individual is united by it to four others, the two which are diagonally opposite it on the other side of the series, and the two adjacent to it on its own side. As the wheel-shaped colonies, Plate I, Fig. 2, are formed, the processes from all the members meet in the center and bind them together.

In all the other salpæ, each individual in the chain is joined on to four others, the adjacent ones on its own side of the chain, and the alternating ones on the opposite side, but instead of being effected by a single process as it is in Salpa pinnata, the union is usually brought about by eight, as is shown in the figure of Salpa scutigera, Plate IV, Fig. 1, and Salpa cordiformis, Plate IV, Fig. 6. We know of no species which stand midway between those of the pinnata group and the ordinary salpæ, and we therefore have no phylogenetic evidence, but it seems probable that Salpa pinnata gives us the primitive method, and that originally a single process joined each salpa on to four others, and that this single process has been gradually converted into eight separate ones. In all cases the processes are primarily ectodermal, and they are shown at an early stage in Salpa cylindrica, in section in Plate XXXVII, Fig. 26.

A chain of salpæ may be compared to two trains of cars on two parallel tracks, placed so that the middle of each car on one track is opposite the ends of two cars on the other track, and each joined by two couplings to the car in front of it on its own track, and in the same way to the one behind it, and also to those diagonally in front of it and behind it on the other track.

In young chains, of all species, on the stolon, the long axes of the salpæ are at right angles to the long axis of the stolon, as if the cars in the two trains were set on end, and this primitive position is, as I have said, persistent in Salpa scutigera and Salpa bicaudata.

Salpa democratica and Salpa tilesii pass through this stage, and before their chains are set free the bodies of all the individuals become inclined in the same direction, as if the cars in the train were pushed over till each one rests against the one in front of it, Plate XLIII, Fig. 1.

This change takes place in such a way that the oral end of the body of each salpa is thrown towards the distal end of the stolon, and in the

species which have just been named this inclined arrangement is persistent.

In still other species, such as Salpa cordiformis, Plate IV, Fig. 6, Salpa runcinata, Salpa africana and Salpa hexagona, the axes of the bodies rotate until they become nearly or quite horizontal and parallel to the axis of the chain, and we thus have two series of salpæ with each one joined at its anterior or oral end to the posterior end of the one next in front of it in the same row, and with the two rows facing each other by their ventral surfaces, and with the middle of the body of each opposite the joint where two in the opposite row are joined together.

While the union between the salpæ in the series is ectodermal, the cellulose mantles, as they grow, usually come into contact, and flattening against each other to help to maintain the integrity of the chain. In chains like those of Salpa scutigera, Plate IV, Fig. 1, and Salpa democratica, Plate XLIII, Fig. 1, the cellulose mantle is in contact, on the sides of the body, with that of adjacent salpæ in the same row, while in all chains the ventral surfaces of the mantles of the salpæ on opposite sides of the chain come into contact. In chains like that of Salpa cordiformis, Plate IV, Fig. 6, where the salpæ in each row are placed end to end, the area of contact between their mantles is increased, as is shown in the figure, by pyramidal processes at the ends of the body. As long as the chain is intact these processes are bent at right angles to the long axis of the body of the salpa, but when the chains are broken up by storms or other accidents the processes gradually straighten out into the long axis of the body, as is shown in Plate III, Figs. 2 and 3, which are dorsal and lateral views of a detached specimen of the aggregated form of Salpa cordiformis.

This brief sketch of the characteristics of salpa chains should be followed by a discussion of their comparative history and of the phylogeny of the chain, but it will be best to postpone this for the present and to treat it in a separate section together with other questions of phylogeny, and we are now prepared to study the details of the process by which the stolon becomes converted into a series of salpæ.

SECTION 3.—*The Segmentation of the Stolon.*

The statement that the stolon becomes converted into, or is morphologically equivalent to, a single row of salpæ placed like a file of soldiers with their long axes vertical or at right angles to the stolon and

with all their dorsal or neural surfaces turned towards its base, and their ventral surfaces towards its tip; that this single row is converted into a double row by the passage of all the odd salpæ to one side and all the even salpæ to the other, and that each salpa rotates on its own axis so that all their dorsal surfaces come to face outwards, and all their ventral surfaces towards those of the opposite salpæ, sounds very simple.

In reality the changes are far from simplicity, and they are extremely difficult to study or to describe in detail.

I have enumerated them in succession and they must be so described, but they all go on simultaneously, and they begin at a very early stage, so that all the space relations of the body of each salpa are changing continually during its development; nor do the changes affect all parts of the body alike, the oral ends with the ganglia being the first to move out into two ranks and the last to rotate.

The oral ends of the bodies, and the ganglia, which are marked *s* in the figures, move from their primitive position on the middle line of the stolon at a very early stage, as is shown in Plate V, Fig. 2, although in Salpa cylindrica, Plate VIII, Fig. 2, long after the aboral or nuclear end of the body has assumed its secondary position, with the dorsal surface and cloacal aperture, g^v, turned outwards or away from the axis of the chain, the ganglion *s* still lies on the proximal surface of each salpa, that is, on the surface which is turned towards the base or proximal end of the stolon, as is shown in the sections of the same stage in Plate XL. In this plate, as in all the others which represent sections of chain-salpæ, the base or proximal end of the stolon is towards the bottom of the page, and the right side of the stolon is on the right side of the figure.

The ganglion of a salpa which has moved to the right is shown at *s* in Figs. 14, 15 and 16, and the ganglion of one which has moved to the left at *s*, in Figs. 19 and 20, and it will be seen that even at this late stage the ganglia on both sides of the series are proximal, although the aboral ends of the bodies have rotated into their secondary position, as is shown in Plate XXXIX, Figs. 10, 11 and 12.

The fact that these changes take place gradually and simultaneously and affect different parts of the body in different ways, renders a clear conception of their character an indispensable preliminary to the study of sections of the stolon, but, unfortunately, the converse is also true, and a clear conception of the character of the secondary changes can only be gained by the study of the details of the process of development.

As two accounts are therefore necessary, I shall first describe the history of the chain-salpa as it would be if there were no secondary changes, and I shall illustrate this first account by imaginary diagrams. I shall also refer from time to time to figures of actual sections which show the points which are referred to, as this will gradually familiarize the reader with the secondary changes, although it will be as well for him to refrain from any attempt to analyze these secondary changes until they are taken up and discussed in due course.

We left the stolon at the stage which is shown in Plate XXXIV, Fig. 1, in which it consists of a tube of ectoderm, *a*, colored violet in the figures; inclosing a nerve tube, *l*, also colored violet, and running along the middle line of the upper surface of the stolon; an endodermal tube, *d'*, colored red; a right perithoracic tube, *h*, and a left one, *g*, both colored green; a string of ova, *n*, colored orange, inclosed in a tubular follicle, *m*, colored blue; and a lower hæmal tube, *i*, and an upper one, *j*, colored yellow. All these structures must be pictured as running along the stolon from its base to the point where it begins to strobilize into salpæ. Three longitudinal sections, with the same colors and letters, are shown in Figs. 4, 5 and 6. Fig. 4, which is near the bottom of the stolon, cuts the ectoderm, *a*, the lower hæmal tube, *i*, and the string of eggs, *n*, in its follicle, *m*. Fig. 5 cuts first, on the right the ectoderm, *a*, then the right perithoracic tube below its lumen, then a number of scattered mesoderm cells, left uncolored, between the perithoracic tube and the area of thickened endodermal epithelium on the right side of the endodermal tube, *d'*, then the ordinary flattened endodermal epithelium on the left, then, in the upper part of the figure, the lower blood tube, *i*, with its endothelium, colored blue, and in the lower part the layer of cellulose, *32*, which fills up all the unoccupied spaces outside the hæmal tubes, as is shown in Fig. 3, and finally, on the left the section again cuts the ectoderm, which, in the section which was drawn, shows by its undulating outline the first trace which I have found of the segmentation of the stolon into salpæ. Fig. 6 is a little higher up, and it passes through the lumen of the right perithoracic tube, *h*, and through mesoderm cells between it and the ectoderm, as well as between it and the thickened endoderm on the right of the endodermal tube. The section passes through the thickened endoderm on the left side of the endodermal tube, but it passes below the left perithoracic tube.

I have not figured a longitudinal section through the nerve tube, Fig. 1, *l*, at this stage, as it would be shown as a simple continuous tube.

Fig. 3 shows the details of a part of the right side of Fig. 1 more highly magnified, but it does not require explanation, since comparison with Fig. 1 will be enough.

As already stated, the first trace which I have found of the strobilization of the stolon into a chain of salpæ is the undulatory outline of the ectoderm, which is shown on the left of Figs. 5 and 6. The peculiar arrangement of the cells seems to indicate that each of the vertical ridges, which are cut transversely by this longitudinal section of the stolon, may be produced by the multiplication of a single row of ectoderm cells, but I have not been able to prove this. In the stolon from which the sections were made the ridges appear on the left side before they do on the right, but the difference is very slight and is perhaps accidental. They first appear in the ectoderm of the middle region of the stolon, and gradually extend up and down towards its neural and germinal surfaces.

Figures 8 and 9 are more highly magnified sections through the left half of the middle region of a slightly more advanced part of the same stolon, and 10 and 11 are through the right half. They are longitudinal sections like 4, 5 and 6, and are, of course, at right angles to the section shown in Fig. 3. They show that the ectodermal ridges are from the first almost exactly equal to each other in width, and that between the ridges the ectoderm grows inwards towards the axis of the stolon, in double folds, which thus form deep vertical furrows separating the ridges from each other. As these folds grow inwards they press upon the right and left perithoracic tubes, h and g, in such a way as to constrict them, and at last to cut them up into series of closed cloacal vesicles, which soon become completely separated from each other, as is shown in the lower part of Fig. 11 at h. My sections indicate that the perithoracic tubes are passive, and that the active agency which divides them up into vesicles is the growth of the ectodermal folds, which, after passing across the perithoracic tubes, begin to push their way in to the lateral masses of thickened endodermal epithelium, as is shown in Fig. 11 and, further advanced, in Plate XXIII, Figs. 7 and 8. The ingrowth of the ectodermal folds goes on, carrying the endoderm before it, as shown in Fig. 9 of Plate XXIII, until the lateral masses of endoderm are folded into a series of vertical pockets, Fig. 9, *28*, which open into the endodermal tube of the stolon, d'. The endodermal pouch on the right side of the stolon, Plate XXIII, Fig. 8, *27*, is the rudiment of the right half of the branchial sac of the chain-salpa; and that on the

left side of the stolon, Fig. 9, *28*, is the rudiment of its left half. The right perithoracic vesicle, Fig. 8, *g*, is the rudiment of the right half of the cloaca; and the one on the left, Fig. 7, *h*, the rudiment of its left half. Plate XV, Fig. 11, is part of a horizontal section on the left side of the stolon, showing these changes in a still more advanced stage. The ectodermal folds have now penetrated to a considerable depth, and they appear from the sections to be the active agents in the formation of the endodermal pockets as well as the cloacal vesicles. At the stage shown in Plate XXXIV the perithoracic tubes lie outside the endodermal thickenings, and the cloacal vesicles have for a time the same positions, as is shown in Plate XXIII, Figs. 7 and 8, *g* and *h*; but as the endodermal pockets, Plate XV, Fig. 11, *28*, grow deeper, their blind ends push past the cloacal vesicles, *h*, on their distal sides, so that the cloacal vesicles themselves come to lie nearer the base of the stolon than the endodermal pockets. Fig. 11 shows this for the left side of the stolon, and the arrangement of the parts is exactly the same on the right side. In this figure, as in all the others, the bottom is proximal or towards the base of the stolon, and the top distal or towards its tip. The changes which we have described are accompanied by important changes in other parts of the stolon, which must now be noticed. They are shown in Plate XV, and also in Plate V, Fig. 1. Like all the subsequent changes, they are of such a character that they cannot be understood or described without sections in at least two planes. I have therefore figured sections at each successive stage from two stolons, of which one was cut parallel to the long axis of the stolon, and transverse to the bodies of the salpæ. A series of these sections is shown in Plate XV, Figs. 1 to 10. Fig. 1 is close to the germinal surface of the stolon; Fig. 2, a little higher up, through the chain of eggs; Figs. 3, 4 and 5, through the lower blood tube; Figs. 6, 7 and 8, through the endodermal tube; Fig. 9, through the upper blood-tube, and Fig. 10, through the neural surface of the stolon. In all these figures the top is distal and the bottom proximal, and the right side of the stolon on the right.

Another stolon was cut into sections transverse to the long axis of the stolon, or parallel to the long axes of the salpæ, but instead of making separate drawings of these sections, I have superimposed the drawings and have thus constructed solid figures. Plate V, Fig. 1, is one of these reconstructions, to show the stage which is shown in Plate XV. It is a dorsal or proximal view and shows the surface which is below in the sections in Plate XV.

When the ectodermal ridges and furrows first appear on the stolon they are restricted to the regions of the perithoracic tubes, but as the folds grow deeper they also extend up and down, and soon completely encircle the stolon, dividing its surface up into a series of complete rings, each of which marks out the body of a salpa. At the stage shown in Plate XV the rings encircle the stolon, as the figures show, although the ectodermal folds penetrate much deeper into the stolon in some places than they do in others. In Plate V, Fig. 1, the shaded area around the periphery shows the extent of the infolded ectoderm, and comparison of this figure with the sections in Plate XV will show its relations much better than words. On the neural surface, Fig. 10, it has grown inwards so far that it has cut up the nerve-tube into a series of ganglia, s, and has pushed down around the ganglion in such a way as to shut it into a pocket of ectoderm, open towards the upper hæmal tube. The active agent in the transformation of the nerve tube into a series of hollow vesicular ganglia seems to be the growing ectodermal fold. Below the ganglion there is a region, cut by section 9, where the ectodermal folds are very superficial and faintly marked; while still lower down in the region of the endodermal tube, d', they are very deep, as shown in Figs. 8, 7, 6 and 5; still lower down, in the region of the lower blood-tube, i, they are slight and superficial, Figs. 4 and 3; while upon the germinal surface of the stolon, Fig. 1, they are very deep, so that they divide the genital string into a series of partially separated segments, which are about half shut in to ectodermal pockets. In general, the ectodermal folds are, as Plate V, Fig. 1 shows, deepest in those regions where the stolon contains solid structures, and most superficial where it is hollow, although each fold forms a complete ring. The structures shown in Plate V, Fig. 1, are the rudiments of a single salpa seen in proximal view, with a circular groove-like body cavity, which opens on all sides into the upper and lower blood spaces, i and j, and communicates through them with the body cavities of the salpæ before and behind it in the series, as is shown in Plate XV, Fig. 4. This body cavity contains a closed vesicular ganglion, Fig. 10, s; two closed cloacal vesicles, a right one, Figs. 7 and 8, g, and a left one, Figs. 5 and 6, h; and two vertical endodermal pockets, which form the rudiments of the right half, Fig. 8, 27, and the left half, Figs. 7 and 6, 28, of the branchial sac. These pockets open into the endodermal tube, Plate V, Fig. 1, d', of the stolon, and through this communicate with each other, and also with the branchial sacs of the salpæ before and behind them in the

series, as is shown in Plate XV, Fig. 7. At the lower end of the body is an egg, *n*, which lies partly in the body cavity and partly in the lower blood-tube, *i*. As Plate XV, Fig. 2, shows, it is not yet completely shut off from the other eggs in the series, although the follicle is beginning to grow in between the eggs. Still lower down, in a pocket of the body cavity, the rudiment of the testis is represented by two folds of the follicle, Fig. 1, *m, m*. Plate XXIII, Fig. 5, shows that the active agency in the segmentation of the genital string is the growth of the fold of ectoderm, *a*, and that this presses in to the genital string and cuts it up just as it cuts up the nerve tube and the perithoracic tubes. The body cavity also contains scattered mesoderm cells, shown in Plate XV, Fig. 11, and the rudiment of the pericardium, Fig. 7, *e*. At this stage this is external to the outer surface of the right endodermal pocket, *27*, and it is probably formed from the cells which are shown in Plate XXXIV, Fig. 3 and Fig. 11, between the right perithoracic tube and the ectoderm. They are shown at a more advanced stage at *e* in Plate XXIII, Fig. 8. I do not know whether there is a left pericardial rudiment or not. In Fig. 5 of Plate XV, shown more enlarged in Fig. 12, there is a rudiment on the left side, exactly like the one shown on the right side in Fig. 7, *e*. Salensky states (2, p. 44) that there is a left pericardial rudiment as well as a right one in the rudimentary ascidiozooid of Pyrosoma, although the definitive pericardium and heart are formed from the right one alone. In Salpa the heart and pericardium are certainly formed from the right one, and if there is a left one it disappears very early, for I found no trace of it in older sections, and after I had drawn Figs. 5 and 11 I discovered reasons for suspecting that I may possibly have drawn an inverted and misplaced section, and that the pericardial rudiment may possibly be only another figure of the right one, which is shown in Fig. 7.

SECTION 4.—*The Development of the Chain-Salpa.*

The vertical endodermal pockets, *27* and *28*, at first open, along their whole length, into the endodermal tube, *d'*, of the stolon, but their upper or *oral* ends soon begin to grow up inside the body cavity of the salpa towards the ganglion, as is shown in Plate V, Fig. 2, so that sections above the level of the endodermal tube show them as closed tubes, Plate XV, Fig. 8, *28*. In Fig. 9 the right side of the section cuts their upper blind ends, while the section passes above them on the left. They

are shown at a younger stage in Plate XXIII, Fig. 10, which is just a little above the level of Fig. 9. In Fig. 9 the pockets, *28*, open into the cavity, *d'*, of the endodermal tube, while in Fig. 10, *28*, they are closed, and separated by the upper blood space, *j*, from the wall, *d''*, of the endodermal tube, *d'*.

These blind pouches grow rapidly and soon reach the top of the stolon, as is shown in cut N, and their slightly dilated oral ends, *27*, *o* and *28*, *o*, lie at the sides of the ganglion, *s*, as is also shown in section in Plate XXIV, Fig. 5, *27*, *28* and *s*. This figure is the uppermost of the series shown in Plates XXIII and XXIV, and in these plates, as well as in those which follow, the capital letters *A–A'*, *B–B'*, etc., indicate the same individual in the series of sections on Plates XXV–XXXIII, the capital letter without an accent marking its right side, and the one with an accent its left side. *A–A'* is the youngest and most proximal salpa, *B–B'* the next, and so on to *N–N'*, which is the oldest and most distal one shown in these plates. *A–A'*, *C–C'*, *F–F'*, *H–H'*, *J–J'*, *L–L'* and *N–N'* are right-hand salpæ, and the alternate ones are left-hand salpæ. As the body of an older salpa is longer than that of a younger one, it is cut by more sections, and is shown in more figures.

In all the figures *27* is the right half of the branchial sac, and *28* its left side. It will be seen from the series of sections in Plate XXIV that the upper or oral ends of the halves of the branchial sac, Fig. 5, *27* and *28*, are dilated, and that, as we follow them down, Fig. 4, they become smaller, until they enlarge again at the level of the endodermal tube, Fig. 2, *d'*, into which they open. Below the level of the endodermal tube they run down for a short distance as closed tubes, Fig. 1, *C*, and these tubes soon lengthen, like the oral ones, and become so long that they reach the genital surface of the stolon; but before this stage the bodies of the salpæ begin to push out of the line of the stolon towards the right and left alternately, and they also begin to rotate on their own axes. The omission of all reference at present to these secondary changes will simplify the description so much that I shall from this point on describe the development of the chain-salpa as if these changes did not occur, and I shall illustrate it by imaginary diagrams. We shall then be the better fitted for going over the subject a second time, as it is actually exhibited in nature, and as it is illustrated by the figures of real sections.

Cut O represents a proximal or dorsal view of a single salpa at the stage just described, in which the endodermal pouches have reached the

neural surface of the stolon or the oral end of the salpa, and have also begun to lengthen towards the genital surface of the stolon or the posterior end of the salpa, carrying with them the two perithoracic vesicles, g and h, and the rudiment of the pericardium, e. The dotted line, i and j, shows the inner edge of the ectodermal fold, and it also of course marks the boundary of the aperture by which the body cavity of the salpa communicates with those of the salpæ before and behind it, while the circle, d', is the boundary of the opening which connects the rudimentary pharynx of the salpa with those of the adjacent salpæ.

The aboral ends of the endodermal pouches, 27 and 28, soon begin to grow backwards, as is shown in cut O, and they carry with them the right perithoracic vesicle, g, and the left, h, and also the rudiment of the pericardium, e, which, it will be seen, is in relation to the right endodermal pouch. The oral ends of the endodermal pouches push in to the middle line of the salpa, as is shown in the diagram, and also in Plate V, Fig. 2, meeting each other on the ventral or distal side of the ganglion, s. As these figures show the proximal or dorsal surface of the salpa, the ganglion is towards the reader, and the oral ends of the endodermal pouches are behind the ganglion. This relation is shown in section in Plate XXXI, Fig. 3, B, where the ganglion is colored purple, and the endodermal pouch, 27, red.

As the endodermal pouches grow backwards (towards the bottom of the stolon and towards the aboral end of the salpa), the wall of each of them unites with the wall of the corresponding perithoracic vesicle, and an opening or gill-slit, which puts the cavity of the vesicle into communication with the endodermal pouch on its own side of the body, is formed at the point of union, as is shown in Plate XXVIII, Fig. 1, c, c'. At C, the gill-slit which connects the right perithoracic vesicle, g, with the right endodermal pharyngeal pouch, 27, is shown, and at C', the one which connects the left perithoracic tube, h, with the left half, 28, of the pharynx. The first trace of the gill-slit is a fold in the endoderm of the dorsal or proximal wall of the pharyngeal pouch, as is shown in Plate XXIV, Fig. 1, 27 and 28.

While this change is taking place the posterior end of the right pharyngeal tube gives rise to a blind diverticulum, Plate V, Fig. 3, q, which is destined to become converted into the œsophagus, stomach and intestine. It therefore represents all the digestive tract except the pharynx, and I shall speak of it as the rudimentary gut. It lies almost directly over or dorsal to the rudimentary pericardium, e, which is thus

partially hidden in a proximal or dorsal view, like the one in the diagram.

These two structures, the rudimentary digestive tract, *q*, and the rudimentary pericardium, *e*, are also shown in Plate V, Figs. 2 and 3. As these figures are not diagrams but reconstructions from actual sections, they are complicated by the secondary changes which have been left out of the diagrams, and Fig. 3 represents a salpa which is about to move to the right, and Fig. 4, one a little older, which is to go to the left, and it will be seen that in both cases the digestive tract and pericardium arise on the right, and in relation to the right pharyngeal tube, whether the salpa is to be a right-handed one or a left-handed one. In Plate XXVII, Fig. 1, *C*, and Fig. 2, *C*, the pericardium, *e*, and the digestive tract marked *g*, are shown, and it will be seen that the digestive tract is proximal or dorsal and the heart distal or ventral. Through an oversight, the rudiment of the digestive tract has been colored green and marked *g*, like the right perithoracic tube in the other figures.

The relations of the various structures in the diagram, cut O, will be made clear by comparison with the following sections, which are, morphologically, in the planes indicated by the horizontal lines.

Plate XXXI, Fig. 3, *B–B′* passes through the ganglion and the oral ends of the pharyngeal pouches. Fig. 2, *B–B′* cuts first, on the left, the left pharyngeal tube in its fold of ectoderm, next the blood space, *j*, through which the body cavity of the salpa communicates with those of adjacent salpæ. Plate XXX, Fig. 3, *B–B* shows the opening by which the right pharyngeal tube, *27*, communicates through the endodermal tube, *d′*, with the left pharyngeal tube, Fig. 2, *B′*, *28*, and also with the pharyngeal tubes of the adjacent salpæ. In Fig. 1 the body cavity communicates with the lower blood-tube, *i*, and the pharyngeal tubes are cut below the level of their openings into the endodermal tube. In Plate XXIX, Figs. 1 and 2, the right half of the body cavity is cut below the lower end of the right pharyngeal pouch, while the left is shown at *B′*. In Plate XXVIII, Figs. 1 and 2, *B′*, the gill-slit through which the left perithoracic vesicle, *h*, communicates with the left pharyngeal pouch, *28*, is shown; while the right half of the body cavity, *B*, is empty in Plates XXVIII and XXIX, and in Plate XXX, Fig. 2, *B*, the right perithoracic vesicle is shown, at *g*, as yet without an opening into the right pharyngeal pouch. Salpa *B–B′* in these figures is a left-handed one, and the lack of symmetry on its two sides is the result of secondary changes, although the sections are still sufficiently like the diagram to be useful for comparison.

Plate XXVI, Fig. 3, *B–B'*, is close to the aboral end of the diagram, and it cuts the two testicular folds, *m*, colored blue, and also the egg, *n*, colored orange, in its follicle.

Cut P is a diagram of a stage a little older, in which the oral ends of the pharyngeal pouches have united with each other on the morphological middle line of the body, ventral to the ganglion, *L*, to form the oral end of the pharynx. This union first takes place at the extreme tips of the pouches, but it runs back rapidly and soon reaches to the endodermal tube of the stolon, *d'*. Plate XXX, Fig. 3, *E'*, *28*, shows the two pouches with their walls fused, but with no connection between their cavities.

At the aboral end of the body the two pharyngeal pouches incline towards each other, and each perithoracic tube has given rise to an outgrowth which extends inwards towards the middle line of the dorsal surface, where it is ultimately to meet and fuse with its fellow from the opposite side to form the atrium. The salpa, which is marked *H–H'*, in Plate XXV, Figs. 5, 6 and 7, and Plate XXVI, Fig. 1, shows the elongation of the perithoracic vesicles, *g* and *h*, towards the middle line of the dorsal surface, and this is also shown at *g'''* in Plate VI, Fig. 1. These various figures also show that the position of the perithoracic vesicles is such, that as they grow towards the middle line they cover up the egg, so as to hide it from direct observation in a dorsal or proximal view. The blind end of the rudimentary digestive tract is now dilated to form the stomach, *p'*, which is joined by a short œsophagus, *q*, to the posterior end of the right pharyngeal pouch.

In the next diagram, cut Q, the oral ends of the pharyngeal pouches have united with each other completely, so that a cross section of this region would now show only a single chamber, and the posterior ends of the aboral pouches are beginning to approach each other, although they have not yet united. The perithoracic pouches have, however, met and united on the middle line of the dorsal surface to form the cloaca, *g'''*, which is also shown at *g'''* in Plate VI, Fig. 2. Plate XXXIII, Fig. 3, *K–K'*, shows the perithoracic pouches, colored green but not lettered in this figure, at the stage when they have met on the dorsal (proximal) middle line, but before their cavities have united. It will also be seen that while the posterior pharyngeal pouches, *27* and *28*, *K–K'*, Plate XXXIII, Figs. 6, 5, 4, 3, 2, 1, and Plate XXXII, Figs. 8, 7 and 6, have nearly met on the middle line, they have not yet united. The digestive tract is now divided into an œsophagus, *q*; stomach, *p'*, and intestine,

p, and the latter now opens into the cloaca, at p''. Plate VI, Fig. 2, is a proximal or dorsal view of a right-hand Salpa pinnata at this stage, showing also beyond it, and partially hidden by it, a left-hand salpa.

In both figures, g is the left and h the right perithoracic vesicle, and g''' the cloaca. Plate VI, Fig. 3, is the opposite or ventral view of the same right-hand salpa, showing the gill-slits or the openings of the perithoracic vesicles, g and h, into the pharyngeal pouches, 27 and 28, and this figure also shows that the posterior branches of these pouches do not yet communicate with each other, although the oral end of the pharynx has been formed by the union of the oral ends of the two pouches, as cuts P and Q show. Plate VII, Fig. 1, is a more highly magnified ventral view of the aboral end of the right-hand salpa shown in Figs. 2 and 3. In this, as in the other figures, 27 is the right pharyngeal pouch, and 28 the left, and g and h are the openings or gill-slits which lead from them into the perithoracic pouches. Beyond or dorsal to the rudiment of the testis, x, is the atrium or cloaca, which is represented as seen through the transparent testis. The space in which the testis lies is to become the cavity of the gill, and, as the figure shows, it is bounded dorsally by the atrium, and at the sides by the pharyngeal pouches. It is not yet closed or shut in ventrally, as it will be when the pharyngeal pouches unite with each other.

The cavity of the gill is also shown, in the same condition, in Plate XXXIII, Fig. 3, K-K', where it is colored yellow. The letter m in this figure lies in the gill, which will be seen to be bounded dorsally by the cloaca, *green*, and at the sides by the *red* pharyngeal tubes 27 and 28, while it is as yet open on its ventral side. A little farther back, Figs. 2 and 1, K-K', it is open both dorsally and ventrally.

In the ventral view, Plate VII, Fig. 1, the pericardial vesicle, e, is shown as a closed hollow vesicle lying against the ventral surface of the posterior end of the right pharyngeal pouch, 27, and, dorsal to it, the postero-median angle of the right pharyngeal pouch gives rise to the œsophagus, q', which leads to the stomach, p', from which the intestine runs towards the dorsal surface to the anal end p'', where it joins the cloaca. In the figure the dorsally placed intestine is represented as seen through the ventral pericardium, e, and the wall of the right pharyngeal pouch. The pericardium is also shown at e in the sections of the salpa, K-K', in Plate XXXII, Figs. 6, 7 and 8, and in Fig. 7, K-K', the œsophagus is shown, colored green, at g, and the cross section of the intestine is also shown in this figure and Fig. 6, colored blue.

The diagram in cut R shows the beginning of the union between the posterior divisions of the pharyngeal pouches, to form the aboral division of the branchial sac. As the diagram shows, this union first takes place some distance from the aboral ends of the pouches, and it gradually extends both backwards and forwards. The series of sections of the salpæ M–M' and N–N', in Plates XXXII and XXXIII, show the branchial sac at this stage. Fig. 6, M–M', of Plate XXXIII, is close to the anterior border of the area of union, and while the two pouches 27 and 28 meet and touch on the middle line, they are not united as they are in M–M' in Figs. 4 and 3, which are sections through the area of union. In Fig. 1, M–M', and in Plate XXXII, Figs. 8 and 7, which is close to the posterior border of the area of union, they are in contact but do not communicate, while in section 6 they appear as two independent tubes, the sections cutting their blind aboral ends. The pericardium of salpa M–M' is shown at e, in Figs. 6 and 5 of Plate XXXII. Fig. 5 cuts the œsophagus at g', and Fig. 3, both the œsophagus g and the intestine h.

Plate VI, Fig. 4, is a proximal or dorsal view of two salpæ at this stage, the left-hand one being in the foreground, and the right-hand one in the distance. Fig. 2 of Plate VII is a distal or ventral view of the left-hand salpa of Fig. 4. It shows the pharynx as a continuous chamber, the two pouches of Fig. 1 having completely united with each other on the morphological middle line.

The next diagram, cut S, shows the young chain-salpa with all its important organs fully formed, seen in dorsal view, as it would appear if there were no secondary changes. It is shown at the same stage in side view in the diagram, cut M, III. Its general anatomy and its relations to the adjacent salpæ are so clearly shown by the two diagrams that explanation scarcely seems to be needed, although I give diagrams of three sections across its body. In these sections the dorsal or proximal surface of each salpa is at the top of the figure, and the right half on the right side. Section T passes through the ganglion L, and below this through the oral region of the pharynx 27, 28. Section U passes through the region where the halves of the pharynx open into the endodermal tube D, and communicate through this with the pharyngeal chambers of adjacent salpæ.

In the plane of this section the ectoderm, a, of adjacent salpæ is continuous, as well as the endoderm b, and their body cavities also communicate with each other in the circular space between the ectoderm and the endoderm. Section V passes through the cloaca; the gill, o; the

aboral region of the pharynx, *27–28*, and the two gill-slits through which the pharynx and the cloaca communicate with each other on the sides of the gill. In cut R the outlines of the gill-slits are represented by dotted lines, while the outline of the cloaca is indicated by a dark line. The rod-like gill, *o*, is also shown in this figure on the middle line between the cloaca above and the pharynx below. Section *V* passes through the same structures, and also through the

CUT T.   CUT U.   CUT V.
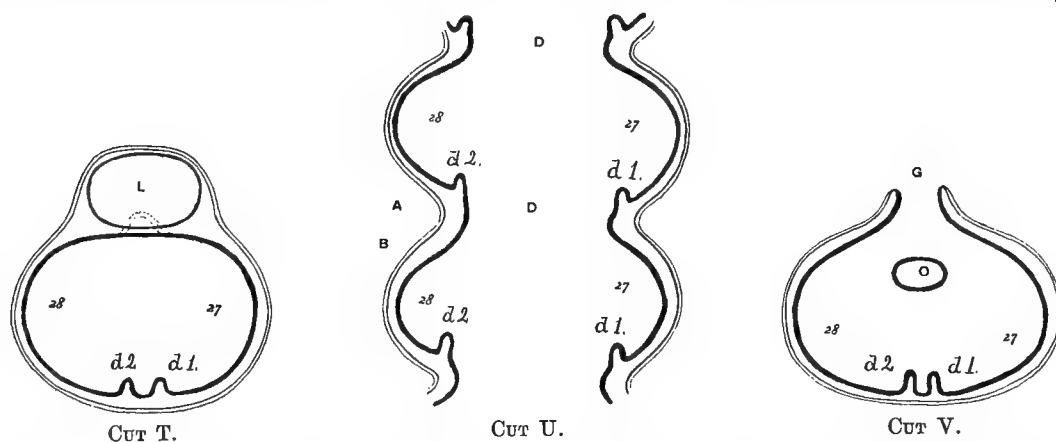
cloacal aperture, *g'''*. It will be seen from these diagrams that the ventral surface of each salpa joins the dorsal surface of the one next it on the distal side, and that its dorsal surface joins it to the ventral surface of the one next it on the proximal side or towards the root of the stolon. The digestive tracts are so joined together that there is a continuous endodermal tube, *d'*, running in an uninterrupted course from the tip of the stolon to the base, where, as is shown in Plate XXI, Fig. 1, *d'*, and also in Plate XIX, Fig. 4, *d'*, and in Plate XXXV, *d'*, this tube joins the pharynx of the solitary salpa on its ventral surface, between the folds of its endostyle, Plates XIX, XX and XXI, *d–d*. The diagrams also show that the pharynx of each salpa is joined on its middle ventral line, between the folds of its endostyle, to the dorsal surface of the next beyond it, so that in this respect the anatomical relation which each one bears to the one next beyond it is precisely the same as that in which the solitary salpa stands to the first one in the series of chain-salpæ.

I have represented each salpa in cut M as if it were joined to the next by an unspecialized connecting tube, but there is no such tube between the actual salpæ, which are separated in the diagram for the sake of clearness. As section *U* shows, both ectoderm, *a*, and endoderm, *b*, pass over from one salpa to the next directly, without the intervention of any connecting tube, and this is shown still more clearly in Plate XV.

SECTION 5.—*The Secondary Changes in the Position of the Aggregated Salpæ upon the Stolon.*

We have now to describe in detail the secondary changes in the position of the salpæ upon the stolon.

As I have already pointed out, these are of two sorts. In the first place, the salpæ in the single series push alternately to the right and to the left, and form two rows instead of one; and in the second place, each salpa rotates on its own axis, so that in all the salpæ the dorsal or neural surfaces, which were originally proximal or towards the base of the stolon, come to face outwards, while the left sides of all the right-hand salpæ and the right sides of those on the left become turned towards the proximal end or base of the stolon.

These changes do not take place in succession, but simultaneously, nor do they take place suddenly after the bodies of the salpæ are fully developed. On the contrary they begin at a very early stage, and they go on gradually while the salpæ themselves are gradually taking shape and undergoing their development and growth. The difficulty in tracing them or describing them is entirely due to this peculiarity, and to the fact that they must be studied in sections. If the successive stages could be dissected out and studied in surface views, the subject would not be obscure or difficult; but it is obvious that sections in a fixed plane, through a series of developing animals which are constantly changing their relative positions, must necessarily be hard to interpret.

If the salpæ first became fully developed while in their primitive or morphological positions, and then after they were fully formed assumed their secondary positions, the case would be as simple as the formation of two files of soldiers from a single file.

If there were a species in which the salpæ completed their development before the secondary changes took place, this species would be the easiest one to understand. There is no such species, and in all which I have studied the secondary changes begin very early, but certain species retain very much longer than others, in the structure of the young chain-salpæ, traces of their primitive position and a record of the path they have followed in reaching their secondary position.

Of the three species which I have studied by sections, Salpa africana is the easiest to understand, Salpa cylindrica next, and Salpa pinnata is the most perplexing. It will therefore be best to speak of these three species in this order.

The stage which is shown in diagram N may be made the starting-point for our study of the secondary changes, although they begin in the actual salpa before this stage is reached. We must picture to ourselves a stolon made up of a series of segments like the diagram, placed in single row so that only one of them is visible when the series is viewed from either end. The digestive tract of each segment communicates with those of the preceding and following ones through the opening d', which thus forms a continuous endodermal tube, running from one end of the stolon to the other, dilated in each salpa into the two pharyngeal pouches *27* and *28*, and constricted, where each salpa joins the next one, to the aperture d'. As is shown in Plates XXIII and XXIV, the body cavity of each salpa is partially shut off by the fold of ectoderm, the inner edge of which is shown in the diagram at i and j; while inside this line the body cavity of each one communicates with those of adjacent salpæ through the blood spaces which are shown in the diagram, and also in Plates XXIII and XXIV at i and j.

These blood spaces therefore form continuous tubes like the endodermal tube d', running from one end of the stolon to the other.

Finally we must note that, since the whole stolon becomes converted into salpæ which are directly connected with each other, as Plate XXIV shows, without the intervention of undifferentiated connecting tubes, it follows that the endodermal tube is, in every section, part of the pharynx of some salpa, and that every part of the blood-tubes is, in the same way, part of the body cavity of a salpa.

Now imagine that these rudimentary, ring-like salpæ move alternately to the right and left, in the directions shown by the arrows, so that they reach the places which they occupy in Plate VIII, Figs. 1 and 2, without breaking loose from their connection with each other in the series. In describing this change it will be best to resolve the arrow into a horizontal and a vertical component, and to picture first the movement outwards, which is shown in diagram W, and then the movement downwards, which is shown in diagram X.

In Salpa africana (and also in Salpa democratica, Salpa cordiformis, and in most salpæ with curled stolons) the animals move almost directly outwards, in the direction which is indicated by the horizontal arrow; in Salpa cylindrica they move outwards and downwards in the direction indicated by the oblique arrow, until they assume the positions shown in Plate VIII, Fig. 2, and finally, in Salpa pinnata, Plate VIII, Fig. 1, the line of movement is rather downwards than outwards in the direction indicated by the third arrow.

CUT W.   CUT X.
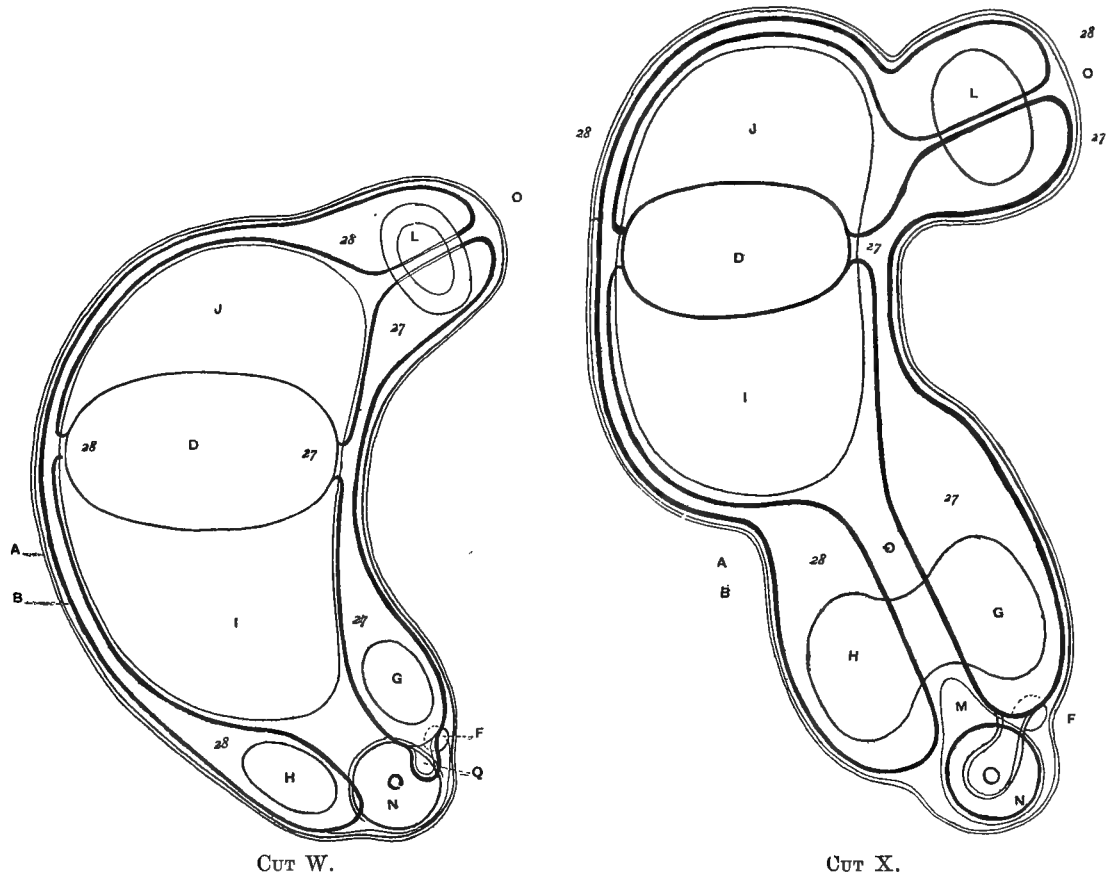
It is plain that, as there are no connecting tubes, the part of the body of each salpa which is joined to those before and behind it must retain its original place in the axis of the stolon, while the only structures which are free to move are those at the oral and aboral ends of the body. The salpa which pushes out to the right will therefore take the shape which is shown in diagram W. The body becomes divided into two distinct regions: 1st, the oral and aboral ends with the ganglion *s*, the reproductive organs *n*, the oral ends 27 and 28, *o*, of the pharyngeal pouches, and their aboral ends 27 and 28, *ab*, with their perithoracic vesicles *g* and *h*; and 2d, the middle region of the body which still maintains its connection with those of adjacent salpæ, and consists of an endodermal tube *d'* and the oral and aboral blood spaces. As the diagrams show, the endodermal tube *d'* retains its connection with the pharyngeal pouches, and at this stage the only communication between the pharyngeal pouches of the two sides of the body is through the endodermal tube *d'*.

In this way each salpa becomes divided into a lateral portion, which ultimately becomes completely shut in by ectoderm and separated from

the adjacent salpæ, and an axial portion in which the communication between adjacent salpæ persists until just before birth, when it degenerates and disappears.

Transverse sections like those from which the figures in Plates VII and VIII were constructed look as if the salpæ had been formed on opposite sides of a connecting tube, and in fact from a physiological point of view this is, practically, the result of the process, for the tube which is shown in these figures lies in the same place as the original stolon, and serves the same purposes in nutrition, although it will be seen that, morphologically, it is in no sense the same as the undifferentiated stolon, but rather the middle regions of the bodies of the salpæ, retaining their original positions after their oral and aboral ends have pushed out of the line. In any transverse section of this tube, at the stages shown in Plates VII and VIII, it is part of the body of a salpa, although it is a part which is destined to degenerate and disappear, just before the birth of the salpæ, after it has served its nutritive purpose.

Since the right and left pharyngeal pouches of each salpa retain for some time the connection with the endodermal tube, d', which is shown in diagrams W and X, it is plain that the anatomical relations of the right half of the pharynx must be different from those of the left, and that those of the left half of a right-hand salpa are like those of the right half of a left-hand salpa. The right pharyngeal pouch, 27, of a right-hand salpa undergoes little change as it pushes to the side, and its direct connection with the right side of the endodermal tube persists, while the oral and aboral ends of the left pharyngeal pouch, 28, of a right-hand salpa are pulled out into long tubular channels of communication with the left side of the endodermal tube.

These peculiar relations are, of course, reversed in a left-hand salpa. Here it is the left pharyngeal pouch, 28, which retains its immediate connection with the endodermal tube, while the communication between the two ends of the right one, 27, is through the long tubes.

If now we imagine each salpa to complete its development in the way we have already described, and then to rotate on its own axis, we shall have a series of salpæ like Plate VIII, Fig. 2, which shows in the foreground the left side of a right-hand individual of Salpa cylindrica, together with that part of the axial tube which pertains to its own body, and in the background the right side of a left-hand salpa partially hidden.

SECTION 6.—*The Rotation of the Salpœ.*

The rotation of the body upon its own axis is a simple process, and the only perplexing complication comes from the fact that the whole body does not rotate at the same time. The rotation begins at the aboral end of the body, and gradually extends along the axis towards the oral end, which rotates last. The way in which it takes place is well shown in cut Y, which is a series of salpæ of Salpa africana, which will also serve to illustrate the simplest method of forming two series from a single row of salpæ, as the process in this species is essentially that which is represented in diagram W.

This figure is a horizontal longitudinal section through a stolon of Salpa africana at the stage in which the salpæ have pushed to the sides and have formed two rows. The lower or aboral ends of their bodies have rotated so that their dorsal surfaces are external, while the upper or oral ends are more nearly in their primitive positions. The salpæ are numbered, in their morphological order, from 1, at the distal end, to 21, at the proximal end, and sections 1, 2 and 3 are shown, more enlarged, in Plate XXXVII, Fig. 26; sections 4 and 5 in Fig. 25; and 6 and 7 in Plate XLVI, Fig. 2. It will be seen that, at the oral end of the body, the ganglia, *s*, are proximal or towards the base of the stolon, and their right and left sides on its right and left.

CUT Y.

(As the series to which these sections belong was begun at the aboral end, the salpæ are placed with their aboral ends towards the observer, and the right side of the stolon, *R*, is therefore on the left of the figure.)

As the stolon of Salpa africana is not straight, but curved in the arc of a circle, as is shown in Plate IV, Fig. 2, a longitudinal section of the stolon will cut successive salpæ at different levels, so that a single section, like the one which is figured, not only shows the relation of the bodies to each other, but also gives what is equivalent to a consecutive series of sections through a single salpa, although the curvature of the stolon is such that no two of them are in strictly parallel planes, and the
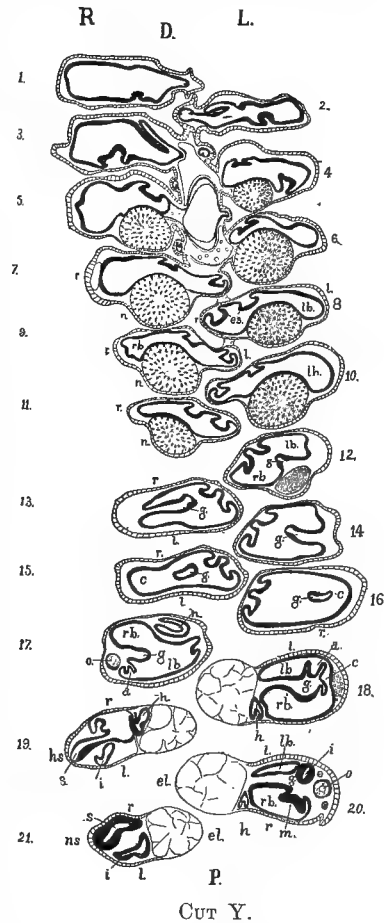
successive sections become more and more oblique as we pass from D to P. The gradual increase in the length of the intervals between the sections is another effect of the curvature of the stolon.

The section which is figured slants upwards distally, so that the animals, *21* and *20*, nearest the proximal end, P, of the stolon are cut through their nuclei and eleoblasts; those which are a little more distal, *15* and *16*, through their branchial sacs, gills and cloacæ; those a little higher up, *10* and *11*, through their ganglia, *n*; *7* is cut through its line of union with the ectoderm of the axial tube; *6*, through its area of communication with the lower blood space; *5*, through its area of communication with the endodermal tube; *4* and *3*, through their communication with the upper blood space; *2*, through the area of union with the ectoderm of the axial tube, and *1*, above this, at the oral end of the body. Between *1* and *7* the salpæ are so crowded that there is no room for the reference letters, and I therefore give on Plate XXXVII and Plate XLVI more enlarged figures of these salpæ, lettered and numbered uniformly with the figures of Salpa cylindrica and Salpa pinnata.

The section numbered *21* passes through the eleoblast, *el*, the stomach, *s*, and the intestine, *i*: the middle plane of its body is at right angles to the long axis of the stolon; the dorsal surface external, and the left side proximal. It is therefore a right-hand salpa, as are all the odd numbers in the series. In the next four sections, 20, 19, 18 and 17, the œsophagus is shown opening at *m*, into the right half, *rb*, of the pharynx, which is separated from the left half, *lb*, by the base of the gill, *g*. In these four sections the heart, *h*, is also shown on the right side of the body. The intestine, *i, 20* and *21*, opens through the anus, *a, 17* and *18*, into the cloaca, *18, i*, on the left side of the gill. The egg, *o*, is shown in *17* and *20*. Figs. 15 and 16 still have their dorsal surfaces outwards, and the middle plane at right angles to the long axis of the stolon, but the endostyle is oblique, its right half in Fig. 16, and its left half in Fig. 15, inclining towards the far side. This is the first indication we meet of the primitive position of the salpæ on the stolon, as we pass from the aboral towards the oral end of the body, and careful examination will show that it is the left half of the odd numbers or right-hand salpæ, and the right halves of the endostyles of the even numbers or left-hand salpæ which push in towards the opposite side. If the series from *14* to *6* be carefully studied, it will be seen that the left halves of all the endostyles finally lie to the left of the middle line, and the right halves of all of them to its right. The series also show that all the hearts, in both right-

hand and left-hand salpæ, are adjacent to the right halves of the endo-
styles, and the study of younger specimens shows that all the hearts and
all the stomachs and intestines are derived from the right side of the
stolon.

In 8, 9 and 10 the ganglion, n, is towards the base of the stolon, and
the right sides of all three salpæ towards the right side of the stolon.
Although these three sections form a double row, as they do at a lower
level, the sections in this plane show that this is only apparent, and is
due to the necessity for room for the great ganglia of the closely packed
series of animals. The figures show that the series is, in reality, a single
row of animals with their neural surfaces towards the base of the stolon,
and with one-half of the pharynx with its half of the endostyle com-
pressed into the space between two other animals, while the other half is
free and enlarged.

In 7, which is shown more magnified in Plate XLVI, Fig. 2, the left
half of the pharynx, lb or 28, and the left half of the endostyle, d, 2,
are obviously on the right of the stolon, although the animal itself is a
right-hand salpa. Its ectoderm is continuous with the ectoderm of the
bottom of the stolon.

Section 6, shown also in Plate XLVI, Fig. 2, is a left-hand salpa at
the level at which its body cavity is continuous with the lower blood
space, ah or i. The right half of its pharynx, 27, and the right half of
the endostyle, d, 1, are separated from the corresponding structures on
the left, 28 and d, 2, by the space i.

Section 4, which is also shown more enlarged in Plate XXXVII, Fig.
25, is a left-hand salpa at the level of its communication with the endo-
dermal tube, d'. This tube is cut obliquely, so that the flat surfaces of
its upper and lower walls are shown as well as its cavity. This figure
shows that the endodermal tube is really part of the pharynx, and that
half of the endostyle, d, 1, lies on one side of it, and the other, d, 2, on
the other.

In section 3, shown also in Plate XXXVII, Fig. 26, the body cavity
communicates with the upper blood space, j, which separates the right
half of the pharynx, 27, from the left, 28, and these two chambers have
here no connection with each other, although still higher up, at the level
of section 2 and section 1, they meet and unite to form the oral end of
the pharynx.

If now we construct in imagination the body of a single salpa from
this series of sections, we shall find that the cavity of the stolon is really

part of its body cavity, and that each salpa is joined on to adjacent salpæ in the way shown in cut M, which is a diagram constructed from sections of Salpa africana.

In Salpa cylindrica the secondary changes take place so slowly that the young chain-salpa becomes fully developed, or nearly so at least, before they are completed, and this species is therefore very well adapted for use as an illustration, and as a key to the more obscure histories of other species.

I have therefore figured a series of transverse sections through the bodies of two salpæ of this species, a right-handed one and a left-handed one, in Plates XXXIX and XL, at the most instructive stage. These figures, like the others, are placed so that the side of the section which is nearest the base of the stolon is at the bottom, and that which is to the right of the axis of the stolon, on the right of the figure. The sections are numbered in succession, so that Fig. 1 is at the aboral end, and Fig. 23 at the oral end of the series.

I have also made from another stolon of the same species at the same stage, another series of sections, at right angles to those in the plates, or transverse to the axis of the stolon, and I have superimposed, with a camera, the outlines of these sections in such a way as to combine them in the solid picture, which is shown in Plate VIII, Fig. 2, which shows in the foreground the left side of a right-hand individual of Salpa cylindrica, together with that part of the axial tube which pertains to its own body; and in the background the right side of a left-hand salpa partially hidden.

Before this figure is described it will be necessary to speak of certain points in which this species differs from all the others I have studied.

In all the species, this as well as the others, the oral ends of the pharyngeal pouches meet and unite on the middle line at a very early stage to form the oral end of the pharynx, as is shown for Salpa pinnata at 27 and 28 in Plate V, Figs. 2 and 3, and also in Salpa pinnata in Plate XXX, Fig. 3, E'.

In Salpa cylindrica, as in the other species, this union at the oral end of the pharynx takes place long before the stage shown in Plate VIII, Fig. 2, is reached, and Plate XL shows that they are united, in Figs. 14, 16, 17 and 18, 27 and 28, for a left-hand salpa, and in Fig. 21 for a right-hand one.

The union between the aboral ends of the pouches does not take place quite so soon, but in all the species which I have studied, except

Salpa cylindrica, it takes place very early; and in Salpa pinnata they have met on the middle line and united at the stage shown in Plate VI, Fig. 4 and Plate VII, Fig. 2, and in Plate XXXIII, *M-M'* and *N-N'*.

In Salpa cylindrica the aboral ends of the pouches remain distinct from each other until an advanced stage of development is reached, Plate XXXIX, *27* and *28*, although they come together on the middle line and are separated only by a thin flat mesentery, which is shown in the sections as a double layer of flattened endoderm cells, running vertically from the gill, *o*, to the middle line of the ventral surface of the pharynx between the folds of the endostyle. The connection between the axial portion of the body and the lateral portion also persists in Salpa cylindrica very much longer than it does in any other species which I have studied.

The position of the endostyle is most instructive in Salpa cylindrica. In Salpa pinnata it does not make its appearance until about the stage shown in Plate VIII, Fig. 1, after the pharynx has assumed its adult form and has entirely lost its connection with the endodermal tube, so that this species tells us nothing whatever about its morphological position, but in Salpa cylindrica it makes its appearance at the stage shown in diagram Q as a thickening or ridge in the ventral wall of each pharyngeal pouch, involving both its aboral and oral portions. As these pouches approach each other and unite, the two folds which are to form the endostyle approach and become parallel at their anterior and posterior ends, while they diverge from each other in the middle region of the body and pass around the endodermal tube.

As they are differentiated at such an early stage in this species, they are of course involved in the secondary changes of position, and they furnish excellent landmarks for studying the character of these changes.

Thus, in Plates XXXIX and XL if we fix our attention on the right half of the pharynx, *27*, and the right half of the endostyle, in Fig. 10, we can trace them through Figs. 11, 12, 14, 15, 16, 17, 18, 19, 20 and 21. This last section is near the oral end of the body, anterior to the ganglion, Figs. 19 and 20, *s*, and we see that the right half of the pharynx of a right-hand salpa is a continuous structure, as it is represented at *27* in cut W.

In a section, which has not been drawn, between Fig. 20 and Fig. 21, it gives rise to the small tube which is shown at *27* in Fig. 21. This tube, which is also shown at *27* in Plate VIII, Fig. 2, is the connection between the right half of the pharynx and the right side of the endo-

dermal tube, d'. The relations of the left half of the pharynx of a left-hand salpa and the left half of its endostyle are, of course, the same; but as the sections which are figured in the plates do not cut the two salpæ at the same points, it will be best to trace these structures also. Starting as before with Fig. 10 of Plate XXXIX, we can follow the left half of the pharynx, 28, and the left half of the endostyle of the left-hand salpa through Figs. 11, 12, 13, 14, 15, 16, 17, 18 and 19, and we find that like the structures on the right of the right-hand salpa they are uninterrupted, and we also notice that in section 15 the left half of the pharynx becomes separated from a small tube, 28, which can be followed through sections 16, 17, 18, 19, 20 and 21 until in section 23 it opens into the endodermal tube, d'. The relations of these parts are thus seen to be exactly as they are represented in cut W 27. The relations of the left halves of the endostyle and pharynx of a right-hand salpa are more complicated, as are also of course those of the right half of a left-hand salpa. Starting as before with Fig. 10 of Plate XXXIX, we will follow the left half, 28, of the right-hand salpa. Figs. 11, 12 and 13 show little change. In Fig. 14 the left half of the endostyle d^2 becomes stretched out towards the far side of the stolon. In Fig. 15, the left pharyngeal pouch 28 has divided into two tubes, one on the right side of the stolon, which comes to an end in Figs. 17 and 18, and one on the left side of the stolon, also marked 28, which can be traced through all the figures, until in Fig. 23 it opens into the endodermal tube, and then goes on, as shown at 28 in Plate VIII, Fig. 2, over the top of the stolon to expand again, in Fig. 21, into the left half, 28, of the oral end of the pharynx. Tracing the right half, 27, of the pharynx of the left-hand salpa in the same way, we find it, in Figs. 14, 15 and 16, passing with the right half of the endostyle on to the right side of the stolon, and after passing over it, reappearing, at 27 in Fig. 18, at the oral end of the pharynx.

These connecting tubes are shown in Plate VIII, Fig. 2, for the right-hand salpa, and it will be seen that they are exactly as they are represented in cut W at 28.

The right half of the pharynx is joined by a short tube, 27, to the right side of the endodermal tube, d', while the oral and aboral ends of the left half of the pharynx have no communication with each other except through the tube, 28, which passes around the stolon, and connects with the left side of the endodermal tube. The left half of the endostyle d^2 bends over onto the far side of the axial tube, as is shown at d^2 in Plate XL, Fig. 14, while the right half d' remains parallel to the

axis of the body, as shown also in the figures on Plates XXXIX and XL. (Through an oversight in the lettering of Fig. 15 of Plate XL, the letter d^1 which is elsewhere used for the right half of the endostyle is used here for the left, and $d, 2$ for the right.)

Soon after the stage which is shown in these figures, the axial portion of the body of Salpa cylindrica becomes entirely shut off from the lateral portion, which becomes converted into a complete salpa, while the axial portion which is morphologically a section taken out of the middle region of its body degenerates and disappears, the endodermal portion being the first to lose its connection with the salpa proper.

In Salpa pinnata these changes take place at a very much younger stage, and for this reason they are difficult to follow without the guidance of Salpa cylindrica.

SECTION 7.—*The Development of the Aggregated Form of Salpa pinnata.*

The direction of the movement outwards by which the single row of salpæ becomes two rows differs in different species. In Salpa africana the movement is almost directly outwards, and Seeliger's figures show that this is true of Salpa democratica also. In Salpa cylindrica the movement is outwards and downwards in the direction indicated by the arrows in cut N, as is also shown by the figure of Salpa cylindrica in Plate VIII, Fig. 2. In Salpa pinnata the movement is more downwards than outwards, as the figures in Plates V, VI and VII show. So far as the secondary changes are concerned, Salpa africana and Salpa democratica are the simplest, and they would be the easiest to understand if the stolon were straight. Salpa cylindrica comes next in intelligibility, but as its stolon is straight it is actually a more favorable subject for study. In Salpa pinnata the secondary changes are most obscure and difficult to trace, but the fact that the series of animals in its straight stolon develop in succession instead of in sets, renders it in many respects the most favorable species to study, and as its secondary changes are fundamentally like those of the other species, they should present no great difficulty after the history of Salpa africana and Salpa cylindrica is understood.

I have selected this species, Salpa pinnata, for the most extended illustration, since, on the whole, it is the most instructive species, and the advantages it affords by its straight stolon and by the gradual development of the salpæ more than compensate for the obscurity which comes from the very early stage at which the secondary changes occur.

A series of sections of this species, parallel to the long axis of the stolon, and transverse to the bodies of the salpæ, is shown in Plates XV, XXIII–XXXIII and XXXVI, XXXVII and XXXVIII.

Another stolon was cut into a series of sections at right angles to those in these plates, but, in order to diminish the number of figures, I have, instead of drawing all these sections, combined them to form the solid pictures shown in Plates V, VI, VII, and Fig. 1 of Plate VIII.

In these figures the numbered cross-lines indicate sections which correspond, or nearly correspond, to the part of the figure which is crossed by the line, but it is very difficult to secure exact correspondence in all cases.

As far as possible, I have shown corresponding stages in the two sets of plates, but the salpæ change so rapidly during their development that the successive stages which are exhibited by one stolon may correspond to the unrepresented intervals between the successive salpæ in another stolon, and I have not been so fortunate as to find two well preserved and perfect stolons which are exactly alike.

The departures from exact agreement are not very great, nor are they of such a character as to perplex the reader, as they relate to slight differences in the shape of the bodies or to slight variations in the relative positions of the organs.

Thus, for example, the line 41 in Plate VIII, Fig. 1, is represented as passing through the process which unites each salpa in one series with the ones diagonally opposite it in the other series. In Plate XXXVIII, however, section 41 actually passes through the bottom of the lower blood space, i, and the connection between the salpæ is cut in sections 50 and 61.

This difference is partially due, perhaps, to distortion caused by hardening and imbedding, but it is chiefly due to the fact that the two specimens are not at exactly the same stage of development, although they are so nearly alike that they can be compared without difficulty.

I shall now describe the two sets of figures in detail. Plate V, Fig. 1, shows a single salpa in the symmetrical position before the secondary changes of position begin. The shaded area shows the extent of the fold of ectoderm which separates this salpa from those adjacent to it, and it will be seen that this fold is most developed where the stolon contains internal organs, and least developed where it is empty.

Thus the testicular folds of the genital string, Plate XV, Fig. 1, m, and the lower portion of the egg, n, are shut into an ectodermal pocket, while a little higher up, Figs. 2 and 3, the ectodermal folds are little more

than undulations in the outline of the ectoderm. In the region of the endodermal pouches, *27* and *28*, the body cavity is very much more shut in, and section 4 of Plate XV cuts the bottom of the left body cavity just above the point where it begins to deepen, while the right half of the section is at a lower level and shows the ectoderm as an undulating line. Section 6 of Plate XV shows the left pharyngeal pouch, *28*, and the left perithoracic tube, *h*, shut in to a deep ectodermal fold, while on the right side of the section the ectodermal fold is cut below the level of the pharyngeal pouch. This section also shows the opening by which the left pharyngeal pouch, *28*,. opens into the endodermal tube, *d'*, and in section 7 the opening of the right pharyngeal pouch, *27*, as well as that of the left, *28*, is shown. Fig. 7 also cuts the pericardium, *e*, and the right perithoracic tube, *g*, above its lumen. Fig. 8 is like Fig. 7 on the right side, except that it passes above the pericardium, but on the left it passes through part of the upper blood space, *j*, and cuts the oral pro-longation of the left pharyngeal tube above its connection with the endo-dermal tube, *d'*. Fig. 9 passes above the left pharyngeal pouch, but it cuts the right one close to its blind end. Finally, section 10 cuts the ganglia, *s*, which are entirely shut in by the folds of ectoderm.

The cuts N and O, on page 80, and the series of sections in Plates XXIII and XXIV, show the gradual extension of the pharyngeal pouches towards the oral and aboral ends of the body. The oral ends, Plate XXIV, Fig. 5, *27* and *28*, have reached the level of the ganglion, and are dilated and much larger than the tubes, Figs. 4 and 3, which connect them with the region of each pouch which opens into the endodermal tube, *d'*, of Fig. 2. At this stage, as in the one before, all the salpæ are exactly alike and symmetrically placed with reference to the bilateral plane of the stolon, but in the next stage, Plate V, Fig. 2, and in all the following stages, the salpæ which move to the right are different from those which move to the left.

Plate V, Fig. 2, shows a salpa which is to pass to the right, at the stage in which the first traces of the secondary changes make their appearance. Like all the other figures in this plate, it is a proximal or dorsal view of a single salpa.

The lower part of the figure is like Fig. 1 in all essential particulars, but great changes have taken place in its upper part. As shown in Plate XXXI, Fig. 3, *B–B'*, the ganglion, *s*, has moved outwards and downwards, and the oral ends of the two pharyngeal pouches have met and united behind or distal to the ganglion, or on what is to become the

ventral surface of the body, to form the oral end of the pharynx.   This communicates directly with the aboral end of the right pharyngeal pouch, *27*, as will be seen by examination of the series of sections of *B* in Plates XXXI and XXX.   At the stage which is shown in Plate V, Fig. 2, the oral end of the pharynx communicates, through a long connecting tube over the upper surface of the stolon, with the aboral end of the left pharyngeal pouch, *28*, but this connecting tube, which, as we have seen, persists in Salpa africana and Salpa cylindrica, Plate VIII, Fig. 2, *28*, until the chain-salpa is perfectly developed, is very transitory in Salpa pinnata, and in salpa *B–B'* in the sections it has begun to degenerate and disappear.   At the level of sections 3 and 2 of Plate XXXI it is shown at *B'*, but in section 1 of this plate there is no trace of it and the body cavity of *B'* is empty.   It reappears again at *B'* in Plate XXX, Fig. 3, and in Fig. 2 the opening of the left pharyngeal pouch, *28*, into the endodermal tube, *d'*, is shown, and still lower down, at *B'* in Plate XXVIII, the communication between the left perithoracic vesicle, *h*, and the left pharyngeal pouch, *28*, is shown.

Plate V, Fig. 3, is also a right-hand salpa, a little older than Fig. 2. Both halves of the body have begun to move downwards, or towards the genital side of the stolon, and the only trace of the connecting tube of Fig. 2 is a short prolongation, *28'*, from the oral end of the pharynx. This prolongation can be traced up towards the top of the stolon for a short distance, but it soon disappears, so that the only communication between the oral and the aboral ends of the left half of the pharynx, *28*, is now through the endodermal tube, *d'*.

Plate V, Fig. 4, shows a left-hand salpa in which the connecting tube has completely disappeared, and the halves of the body have moved down so far that the ganglion, *s*, is on the level of the ectodermal tube, *d'*, as is also shown in the section of the salpa *E–E'* in Plate XXX, Fig. 2. Above the endodermal tube the body cavity is now empty, as shown at *E–E'* in Figs. 1, 2 and 3 of Plate XXXI, and there is no trace of the connecting tube.   The greater part of the right pharyngeal pouch, *27*, now lies below the level of the endodermal tube, *d'*, and its aboral dilated end is joined to the right side of the endodermal tube by a constricted tubular prolongation, which is shown, in section at *E*, in Figs. 1, 2 and 3 of Plate XXX.   Fig. 3 cuts it at *E* below the level of the endodermal tube, while Figs. 1 and 2 show its opening into this tube.   In Plate XXIX, Fig. 2, it is cut at *C*, as it begins to expand, and at *C* in Fig. 1 its communication with the right perithoracic vesicle, *g*, is shown, as it is

also at C in the figures in Plate XXVII. In Plate XXVII, C, the pericardium, e, is shown in Figs. 1 and 2, and also the diverticulum, g, which is to give rise to the digestive tract. Through an oversight, this is colored green in the figures instead of red. It will be seen from Figs. 3 and 4 of Plate V that the left pharyngeal pouch of a salpa which goes to the right, Fig. 3, like the right one in a salpa which moves to the left, moves much further from its primary position than the other pouch, and becomes correspondingly reduced in cross section, as is shown by the sections which we have just examined of the right pharyngeal pouch of a left-hand salpa. The very small tubular upper part of this pouch, shown in section in Plate XXX, Figs. 1 and 2, E, is the same as the connecting tube, which is shown, at a very much older stage, in Salpa cylindrica, at 27, in Plate VIII, Fig. 2. In Salpa pinnata, as in Salpa cylindrica, it connects the dilated aboral end of the right-hand pouch of the left-hand salpa, Plate V, Fig. 4 (or the left-hand pouch of the right-hand salpa, Plate VIII, Fig. 2), with the corresponding side of the endodermal tube, but in Salpa pinnata it soon degenerates and disappears, as will be shown soon, while it is present in advanced embryos of Salpa cylindrica.

We have now to examine the sections through the left half of the left-hand salpa, shown in Plate V, Fig. 4. Plate XXX, Fig. 3, E' shows the union of the oral ends of the two pharyngeal pouches.

Figs. 2 and 1, E', show the communication between the left one, 28, and the endodermal tube, d', and they also show the ganglion s, proximal or dorsal to the endodermal pouch and at the level of the connecting tube. In Plate XXIX, Fig. 2, c', the section cuts the left perithoracic vesicle, h, and the left pharyngeal pouch, 28, just below the endodermal tube. C' in Fig. 1 shows the communication between these two organs, as does also c' in Plate XXVIII, Figs. 1 and 2; c', in Fig. 3 of Plate XXVII, cuts the aboral ends of the pouch and vesicle, and c' in Figs. 2 and 1 passes below these structures.

It will be seen by comparing Plate V, Fig. 3, with Fig. 4, and by the examination of the sections, that the pericardium, e, is always in relation to the aboral end of the right pharyngeal pouch, and that the digestive organs always arise from this pouch, whether it is the larger, as it is in a right-hand salpa, Fig. 3, or the smaller, as it is in a left-hand salpa, Fig. 4.

The next figure, Plate VI, Fig. 1, is a dorsal or proximal view of a right-hand salpa at the stage which is shown at H–H' in the sections on

Plates XXV-XXXI. The structures which are involved in the formation of the body proper have moved down onto the lower surface of the axial tube, and are beginning to approach each other on the middle plane of the body, which lies pretty nearly in the imaginary line between the letter *n* and the Fig. 27. This is a most important stage, and its careful examination is essential to a comprehension of the development of Salpa pinnata. The course which the ganglion has taken in its migration from its primitive position to the one which it occupies at this stage will be understood from the study of the figures on Plate V.

In Fig. 1 all the ganglia are on the middle line of the stolon and in a row, as is shown in Plate XV, Fig. 10. In Fig. 2 those which belong to right-hand salpæ have moved to the right, and those which belong to left-hand salpæ to the left, although they are still near the top of the stolon, as is shown in Plate XXXI.

In the next stage, Fig. 3, they have begun to move down towards the lower surface of the stolon, although they are still above the level of the endodermal tube, while in Fig. 4 they have reached the level of the endodermal tube, as is shown in Plate XXX, Fig. 2, at *F* for a right-hand salpa, and at *E'* for a left-hand one. Finally, in the stage shown in Plate VI, Fig. 1, they are near the level of the bottom of the stolon, as is shown for a right-hand salpa in Plate XXVIII, Fig. 1, *H*, and for a left-hand salpa in Plate XXVI, Fig. 2, *G'*. As the salpa grows older the ganglion moves further and further from its primitive position, as is shown by comparing the figures in Plate VI, 1, 2 and 4, Plate VII, 3, 4 and 5, and Plate VIII, Fig. 1.

Returning now to Plate VI, Fig. 1, it will be noticed that the extension of the two pharyngeal pouches downwards has resulted in the separation of each pouch into a small connecting tube and an expanded end within the body of the salpa. One of these connecting tubes, *27*, joins the right pharyngeal pouch to the right side of the endodermal tube, while the other, *28*, joins the left one to the left side of the endodermal tube. The latter is the same as the one shown at an earlier stage for a left-hand salpa at *27* in Plate V, Fig. 4, and is also the same as the lower part of tube *28* in Plate VIII, Fig. 2, while the former, *27*, is the same as tube *27* of Plate VIII, Fig. 2.

The three upper sections through Plate VI, Fig. 1, hardly call for explanation, although examination of the sections shows that an important change, the obliteration of the folds of ectoderm between the salpæ, has already begun in this region, and at a later stage the ectoderm of all

the upper part of the axial tube becomes thin and flat and loses all traces of segmentation.

Section H-H' of Plate XXX, Fig. 1, cuts the left connecting tube, H', at its opening into the endodermal tube. The section also cuts the lower wall, d'', of the endodermal tube, crosses the upper part of the lower blood space, i, and cuts the right connecting tube, H, 27, just below its opening into the endodermal tube.

Section H of Plate XXIX, Fig. 2, cuts the oral end of the pharynx and of the ganglion, s, but the left half of the section is not shown in the figure, although F' shows what it would be if it were present. In Plate XXVIII, H, sections 1 and 2 pass through the ganglion, s, and the oral end of the pharynx, 27, and the sections in Plate XXVII, H, cut the right pharyngeal tube below the ganglion.

The section H-H', in Plate XXVI, Fig. 2, cuts the left connecting tube at H' just before it begins to expand in the body of the salpa, and it also passes through the lower blood space, i, close to its floor. In Fig. 1, H-H', the right pharyngeal pouch, 27, and the right perithoracic tube, g', are cut through the aperture or gill-slit which unites these cavities. The left pharyngeal tube, 28, is much flattened dorso-ventrally, as is also the left perithoracic vesicle, and this latter is much elongated towards the middle line of the dorsal surface. In Plate VI, Fig. 1, this elongated portion of the left perithoracic vesicle is marked g'''. It is the cloacal diverticulum shown at G''' in cut O. In the diagram the cloacal outgrowths from the two perithoracic vesicles are represented as equal, but in Salpa pinnata the one which is derived, in the right-hand salpa, from the left vesicle, Plate XXVI, Fig. 1, H', h, and in the left-hand salpa, Fig. 1, G, from the right vesicle, g, appears much larger than the other in sections. This section also shows on the dorsal surface, between the two perithoracic vesicles, the two folds of the follicle which are to give rise to the fertilizing duct of the egg, and, nearer to the ventral surface, between the pharyngeal pouches, the single fold which is to give rise to the testis.

Plate XXV, Fig. 6, H-H', shows the egg, m, in its follicle on the middle line of the dorsal surface between the perithoracic tubes g and h, and Fig. 4, H-H', shows on the left the blind end of the left pharyngeal tube and the aboral surface of the left perithoracic tube. On the right the section passes through the digestive tract, g', and the pericardium, e, which is on the ventral surface of the right end of the body, and so directly behind the digestive tract that it is completely hidden behind it in a dorsal view like Plate VI, Fig. 1.

Fig. 2 of Plate VI is a dorsal view of a right-hand salpa and the tubes which connect it with that part of the axial tube which pertains to its body, while beyond it and partially hidden behind it is the body of a left-hand salpa.

Fig. 3 is a ventral view of the same two salpæ, but as this second figure was reconstructed from the same set of sections, it is not reversed, but the left-hand salpa is on the left side as it is in Fig. 2. In effect, Fig. 3 is Fig. 2 as it would appear under a microscope when focused down to its far side.

Sections of a right-hand salpa at nearly the same stage are shown in Plates XXXII and XXXIII at J–J', and sections of a left-hand salpa in K–K'. The planes of these sections are shown by the cross lines in the figures. The salpæ shown in the sections are a little younger than those from which the solid figures were reconstructed, but the difference is not very great.

In Plate XXXIII, Fig. 9, cuts, on the right side below the level of the endodermal tube, the tubes which connect it with the right halves of the pharyngeal pouches; all the tubes on the right side of this figure belong to the right halves of the bodies, but they belong alternately to right-hand and left-hand salpæ, while all those on the left, cut at the level of their openings into the endodermal tube, belong to the left halves of the bodies. Fig. 8, K–K', cuts the right and left connecting tubes of the left-hand salpa, as is shown by the line K, 8, in Fig. 3, while Fig. 8, J–J', cuts, as the line J, 8, in Fig. 2 shows, the left connecting tube of the right-hand salpa at J', while on the right it cuts the oral end of the pharynx and the ganglion.

Fig. 7, K–K' cuts, on the left, the left-hand connecting tube of the left-hand salpa at 28 just before it expands into the pharynx, while on the right at K it cuts the right connecting tube of the same salpa. The right-hand salpa, J–J', is not shown in Fig. 7, but L–L' is essentially like it, as is J–J' of Fig. 6. As the line J, 7, in Fig. 2 shows, this section cuts the left pharyngeal tube of the right-hand salpa twice, once at the extreme left of section L', and again near the middle of the section. In Fig. 6, J–J', however, the left pharyngeal tube, 28, is cut only once. The right end of section 7, J, cuts the oral end of the pharynx at 27 and the ganglion at s.

PART TWO.

THE SYSTEMATIC AFFINITY OF SALPA IN ITS RELATION TO THE CONDI-
TIONS OF PRIMITIVE PELAGIC LIFE; THE PHYLOGENY OF THE
TUNICATA; AND THE ANCESTRY OF THE CHORDATA.

CHAPTERS VI, VII, AND VIII.

CHAPTER VI.

THE SYSTEMATIC POSITION OF SALPA.

SECTION 1.—*The Evidence that Salpa is descended from a Fixed Form.*

I formerly believed that Salpa is the modern representative of an ancient Tunicate stem which has been pelagic throughout its whole history, and has been evolved at the surface of the ocean from an ancestor something like the modern Appendicularia; and that the group has nothing in common with the fixed Ascidians except a common descent from this ancient form.

While I still regard Appendicularia as the starting-point, I now feel confident that in all other respects this view is wrong, and that the facts force us to believe that Salpa is a modified descendant from a fixed form; that it owes nearly all the distinctive peculiarities of its structure to a sedentary life; that its adaptation to a free life at the surface is secondary and comparatively recent; and that its only connection with Appendicularia is through this fixed ancestral form. It is possible, and I think probable, that this fixed form was not identical with any Ascidian which now exists, and it may have lacked some peculiarities which are shared in common by all modern Ascidians, but it must have been either a fixed Ascidian, or else an ancestor of the fixed Ascidians, with their habit of life and with essentially their structure.

The facts which have forced me to abandon my original opinion and to substitute the view which has just been outlined are these. In the first place, comparative anatomy forces us to believe that the atrium of Salpa is identical with the perithoracic and atrial chamber of ordinary Ascidians, and the facts of embryology show beyond question that this is a real homology.

In the ordinary Ascidians this system arises in the embryo as two ectodermal invaginations, one on each side of the body at some distance from the middle line, which grow inwards towards the pharynx, and become the perithoracic vesicles, which ultimately establish a communication with the pharynx through the gill-slits. The two perithoracic vesicles or lateral atria approach each other on the middle line, dorsal

to the pharynx, and unite with each other to form the median atrium or cloaca, which opens to the exterior on the dorsal middle line through the atrial aperture. This aperture is, morphologically, the two original spiracular openings of the perithoracic invaginations, and in most cases these openings move towards each other until they meet and unite to form the single median atrial aperture. In some cases, however, they close up and disappear, while the atrial aperture breaks through as a new opening on the middle line. In the latter case, however, it is plain that we have to do with secondary changes, and the replacement of a circuitous ancestral history by a more direct mode of development. This secondary history is exhibited by Salpa, as I have shown already. To recapitulate briefly, we have in the embryo of the solitary form, cut A, p. 29, first, a pair of lateral perithoracic involutions from the surface of the body. Then, as Plate XII shows, these involutions, Fig. 1, g'', extend towards the middle line and meet to form the median atrium, Fig. 2, g''', and also extend downwards towards the rudiment of the pharynx as a pair of perithoracic tubes, Fig. 4, g' and Fig. 5, g, which, at the stage shown in Plate XII, end blindly. Cut B on p. 29 shows these structures at this stage in a vertical transverse section.

In the embryo which is shown in Plate XIII, the spiracular openings of the perithoracic tubes have closed, and the external portions of these tubes have moved towards the middle line, Fig. 6, g'', where they meet above the median atrium, Fig. 7, g''', from which the perithoracic tubes, Fig. 8, g^{IV}, are continued down towards the pharynx, although they still end blindly, as the vertical section of the same embryo, on p. 30, shows.

In the embryo shown in Plate XIV they open into the pharynx by a single large aperture or gill-slit, g^{IV}, on each side of the middle line. In this plate, Figs. 3 and 4 cut the perithoracic tubes above the median atrium; Fig. 5 cuts the median atrium; Figs. 6 and 7 cut the tubes at lower levels, and Figs. 8 and 9 pass through the pharynx, c. Cut D on p. 31 is a vertical section of the same embryo. Finally the median atrial aperture, Plate XXXV, g^v, is formed as a new opening on the dorsal surface in the way which is shown in Plate XVII, Figs. 6 and 7.

In the aggregated salpa the atrial structures are formed in essentially the same way. On each side of a cross-section of the stolon, Plate XXXIV, Fig. 1, there is a perithoracic tube, g and h, which probably arises at the base of the very young stolon, Plate XX, Fig. 3, as an involution of the ectoderm, although as Plate XXI, Fig. 7 shows, the

external opening is soon lost. Folds of the ectoderm of the stolon, Plate XXXIV, Figs. 9, 10 and 11, *g* and *h*, soon divide each tube up into a series of vesicles, one on each side of the body of each salpa. Each perithoracic vesicle, Plate XXVI, Fig. 3, *E-E'*, *g* and *h*, now acquires an opening or gill-slit by which it communicates with its own half, 27 and 28, of the pharynx, and they also become produced towards the middle line of the dorsal surface, Fig. 1, *H–H'*, *g* and *h*, where they meet, as shown at *g* and *h* in Plate XXXIII, Fig. 3, *K–K'* and *N–N'*, and ultimately unite to form the median atrium, Plate XXXVI, Fig. 6, *g'''*, which afterwards acquires a median dorsal aperture, Fig. 5, *g*ᵛ.

Writers on the embryology of Salpa and allied animals have involved the history of the atrial system in unnatural obscurity, for its origin in the salpa embryo and in the aggregated salpa is in perfect accordance with the teaching of comparative anatomy, and quite irreconcilable with any view except the one which regards these structures in salpa as strictly homologous with the median and lateral atria of ordinary Ascidians.

It will be necessary to discuss in a later chapter the various views of the writers on the origin and homology of the atrial structures of Salpa, but at present this would lead us too far from our subject.

I have shown in another place, p. 35, that, as Leuckart pointed out long ago, the atrial aperture of salpa is much nearer the mouth when it first appears than it is later, and that in this respect the ontogeny of salpa exhibits evidence of an Ascidian-like stage in its ancestry. The compactness of the ganglion of salpa, as contrasted with the elongated central nervous system of primitive chordata, and its position between the two apertures of the body, are also features of resemblance to the Ascidians; and while there are now no traces, at any stage of its development, of numerous stigmatic gill-slits, like those of Pyrosoma, I shall show soon that there is indirect evidence that they at one time existed in the ancestors of salpa, which are, in this respect, Pyrosoma-like.

The muscle bands of Salpa are easily intelligible as modified oral and atrial sphincters, and they are distinctly more irregular in the young than they are in the adult. In the young aggregated Salpa cylindrica, Plate VIII, Fig. 2, the fourth and fifth body muscles are clearly shown to arise as branches from an atrial sphincter, and Fig. 1 shows one of the body muscles arising in the same way in the aggregated Salpa pinnata.

The peculiar anatomical relations of the pharynx and atrium of Ascidians are generally and justly regarded as modifications which were

gradually added on to the primitive Tunicate type, as adaptations to a sedentary life. . If Salpa has been evolved from a swimming ancestor like Appendicularia through an uninterrupted series of free pelagic stages, we can give no explanation whatever of its Ascidian type of structure, while this is perfectly intelligible on the view that it is a modified Ascidian.

SECTION 2.—*Views on the Relationships of the Swimming Tunicates.*

We must now discuss its systematic relation to the other swimming Tunicates, and the nature of its relation to the Ascidians.

The Tunicates which are most like salpa are Pyrosoma, Anchinia, Dolchinia, Doliolum, and Octacnemius. We know too little of Octacnemius to make much use of it, and if it is related to salpa at all it is not a stem form, but a salpa which has been secondarily modified, so we need give it no more attention at present. If Dolchinia is not a Doliolum, it is so near to Doliolum that it throws no new light on the origin of Salpa.

We do not know the whole life-history of Anchinia, but our knowledge of it is sufficient to show the justice of the opinion which, so far as I am aware, is universally accepted, that it is very closely related to Doliolum, and our comparison may therefore be narrowed down to Pyrosoma, Salpa, and Doliolum, including with the latter Anchinia and Dolchinia.

The student of the recent literature on the systematic relationships of these three groups finds total confusion and irreconcilable contradiction.

I shall not attempt to discuss all the opinions on record, as three selections are enough to show how little is definitely established. I shall therefore confine myself to the views of Grobben (Doliolum. Arbeiten Zool. Inst., Wien, IV, 2, 1882), Uljanin (7), and Herdman (14), and before I discuss the statements of these authors I will state as briefly as possible the distinctive characteristics of each.

Grobben holds (p. 67) that Pyrosoma, Doliolum and Salpa are very closely related; that they form a natural group, and that they have been derived from the *Compound Ascidians*, through a Pyrosoma-like ancestor. So far as they are contained in this outline, I myself accept Grobben's views, except that I doubt whether the Ascidian ancestor had the distinctive characteristic of any modern group of Ascidians.

Uljanin believes (p. 123) that we have in Salpa, Doliolum, and Pyrosoma, representatives of three distinct and independent lines of descent from the *Simple Ascidians*, from which Salpa and Doliolum are directly although independently descended, while Pyrosoma is a modified *Compound* Ascidian, and is, together with all the Compound Ascidians, descended from the Simple Ascidians.

Uljanin gives a genealogical tree of the Tunicates, which, so far as the forms which we are now examining are concerned, is like this—

Pyrosoma   Doliolum   Salpa

Compound Ascidians

Simple Ascidians

Appendicularia
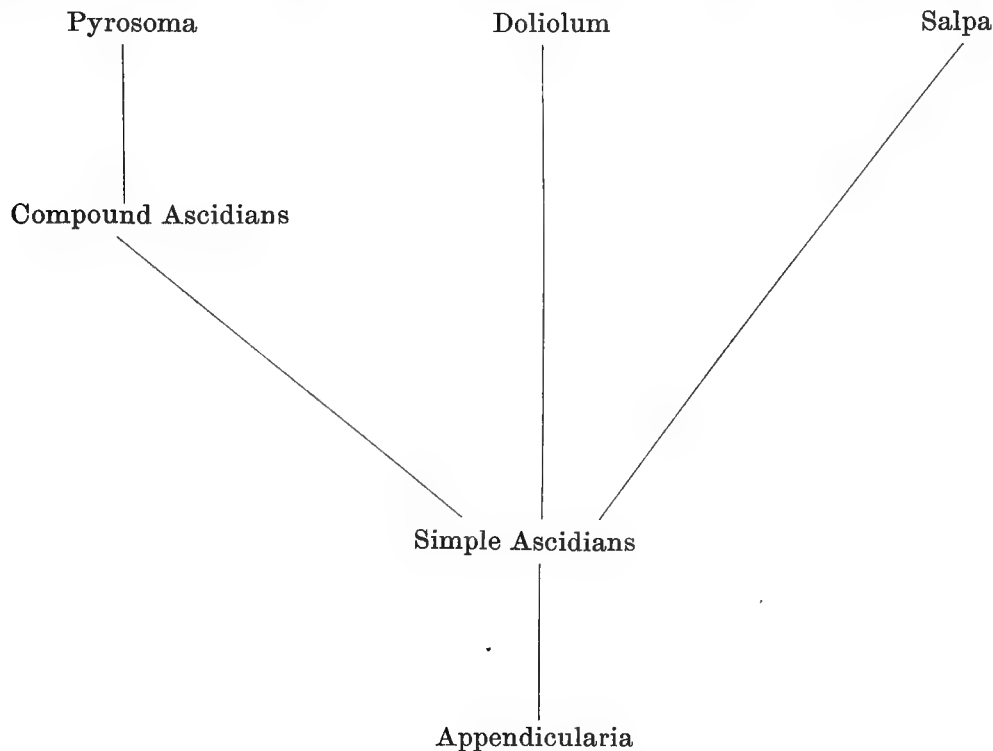
Grobben and Uljanin agree in the opinion that Salpa, Doliolum, and Pyrosoma are all of them descended from Ascidians, but here the agreement ends, while Herdman, p. 124, rejects this very point, and holds that Salpa and Doliolum are descended directly from Appendicularia, and that they have nothing else in common with the Ascidians. He believes that Salpa and Doliolum are closely related, and that they form together a natural group, while Pyrosoma belongs to another line entirely, and is descended from a Compound Ascidian ancestor.

While these views do not exhaust all the permutations and combinations of the three factors, they are enough to show the absence of a common ground for comparison, although it should not be difficult to place the subject upon a sound and permanent basis, as the forms which are to be considered are so very few and our knowledge of them so complete.

SECTION 3.—*Salpa and Doliolum.*

First, as regards the comparison between Salpa and Doliolum, are we to believe with Uljanin that they are widely separated, or with Herdman and Grobben that they are closely related?

To my mind there is no room for doubt. Unquestionably Doliolum, Anchinia, and Salpa are more closely related to each other than they are to any other Tunicate except Pyrosoma.

I have already shown, page 9, that the contrast in the muscle bands upon which the groups Cyclomyaria and Desmomyaria are based has no existence. In all Doliolums some of the muscle bands are imperfect rings; in many species of Salpa the oral and atrial muscles are perfect rings, and in the most common and best known species of Salpa, the solitary Salpa democratica, most of the body muscles are as perfectly closed dorsally and ventrally as' the rings of Doliolum. Anchinia, at least, is only by courtesy a Cyclomyarian, for it has no circular muscles except the oral and atrial sphincters, as the figure of the sexual animal given by Kowalevsky and Barrios (4), Plate III, Fig. 8, clearly shows.

The groups Cyclomyaria and Desmomyaria are then purely artificial and without scientific value.

Uljanin lays stress upon the presence of a tailed larval stage in the embryo of Doliolum and its absence in Salpa, but a comparison of the Doliolum larva which he shows in his Plate V, Fig. 1, and which I have copied in my Plate VIII, Fig. 3, with the embryo of Salpa hexagona which is shown in my Plate III, Fig. 4, will show that the eleoblast k of the salpa embryo is a true tail, bearing exactly the same anatomical relations to the body as the tail of the doliolum larva. In my account of its minute structure, page 38, I have shown that sections prove it to be, without question, a degenerating larval tail.

Uljanin says that he regards Salpa as standing alone among the Tunicates, and that its resemblance to Doliolum is superficial and due to secondary adaptation, but he gives no valid reason for this opinion. He says that the anomalous fœtal development of Salpa, and the part which, according to Salensky, the tissues of the mother-organism take in the construction of the embryo, show that it is very different from all other Tunicates and render it difficult to place in the system. I have shown that the development of the salpa embryo, while very remarkable indeed, is by no means totally anomalous, and while we know nothing of the way its fœtal mode of development and its placenta were acquired, they

must have been acquired at some period in its history, and I do not see that Uljanin comes any nearer to an explanation of its peculiarities by tracing it back to the Ascidians along an independent line. So far as I know, nothing in the history of the Ascidians helps us to understand these peculiarities of Salpa, and Uljanin's view accomplishes nothing except to force him to seek for a secondary explanation of the conspicuous and undeniable resemblances between Doliolum and Salpa.

As Grobben says, page 67, the shape of the body in Salpa and Doliolum, the situation of the mouth and atrial aperture at opposite ends, the arrangement of the muscle bands around the barrel-shaped body, and the free-swimming habit, are in themselves very conclusive evidence of their affinity, and while it is quite true that, as Uljanin points out, these adaptations to similar conditions of life might have been independently acquired, there is no good reason for thinking that this has happened, for they exhibit both superficial and fundamental similarity of structure. Uljanin indeed holds that the gills of Salpa are not homologous with those of Doliolum. If by this statement he means that the rod which usually, in Salpa, goes by the name of "gill" is not the same thing as the branchial slits or stigmata of Doliolum, his objection is unworthy of consideration, for no one has ever seriously proposed any such homology, although the "gill" of Salpa has its homologue in Doliolum, as in Pyrosoma and the Ascidians, in the dorsal lamella. If he means that the two apertures by which the pharynx of Salpa communicates with the atrium are not homologous with the branchial slits of Doliolum, I can only quote my observations, already detailed, which show that the gill-slits of Salpa are strictly homologous with those of the Ascidians. I shall soon examine Uljanin's statement that the atrium of Doliolum is not homologous with the atrium of Ascidians.

SECTION 4.—*Salpa and Pyrosoma.*

I think that we may safely assume, as the first step in our comparison, that Doliolum and Salpa are closely related, and we come now to the question whether Pyrosoma is closely related to Doliolum and Salpa as Grobben believes, or is a swimming Tunicate of very different origin, as Herdman and Uljanin believe.

Since it was first pointed out by Huxley (Remarks upon Appendicularia and Doliolum, p. 602), the affinity between Pyrosoma and Doliolum has received general acceptance, and Grobben, p. 68, has shown that there is a very close anatomical agreement between them.

Uljanin (7), p. 124, characterizes the method of comparison which Grobben employs as irrational, inasmuch as he bases it upon purely imaginary changes in the anatomy of the fully developed animals, and makes no use of embryology.

He says that Grobben overlooks the fact that the atrium or cloaca of Doliolum does not correspond to that of Pyrosoma and the other Tunicates, inasmuch as there are, in the Doliolum embryo, (p. 67) no perithoracic tubes like those of the Ascidians, since the cloaca of Doliolum is formed as a single median unpaired invagination of the ectoderm.

It is not impossible that there may be a pair of lateral perithoracic tubes in Doliolum before the median cloaca and its aperture are formed, for his observations were made upon entire embryos, and no sections of the structures in question are figured. In Anchinia, which Uljanin justly regards as the nearest relation of Doliolum, Barrois has shown (10), pp. 226, 230, 236 and 242, that the atrial structures of the sexual animals arise in the buds as paired lateral ectodermal invaginations, and that their history is exactly the same as the primitive history in the Ascidians, so that Uljanin must either deny the homology between the atrium of Doliolum and that of Anchinia, or else he must recognize its homology with that of the Ascidians and Pyrosoma. Even if his observations are accepted as final, his deduction by no means follows.

In some echinoderm larvæ the cœlomic pouches separate from the gut before they separate from each other, while in most cases they are distinct from each other before they become separated from the gut, yet all embryologists regard them as homologous, and it is vastly more probable that the ectodermal rudiments of the perithoracic tubes of Doliolum meet on the middle line before the invagination takes place than that the atrium of Doliolum is a new structure.

While history gives ample reason for his statement, p. 124, that the facts of comparative anatomy may be distorted or misrepresented, all naturalists know that anatomy often proves homology and furnishes a key to embryology. Thus mammalian teeth and the flat bones of the mammalian cranium are held to be dermal scales, although mammalian ontogeny gives no record of their phylogeny. So too the mammalian body cavity is held to be a series of cœlomic pouches from the gut, and the mouth of a starfish is held to be strictly homologous with the mouth of a sea-urchin, although in the one case it is the same as the larval mouth, while in the other it breaks through on the left side of the larva.

In all these cases we reconstruct the primitive ontogeny from the

evidence of comparative anatomy, and explain modern ontogeny as the result of secondary modification.

Comparative anatomy shows clearly that the atrium of Doliolum is homologous with that of Pyrosoma and the Ascidians, and the onto-genetic history in Doliolum does not present any great difficulty.

I shall have to refer again further on to the nature of the evidence from embryology, but I think all morphologists agree that when organs or animals which are shown by their anatomy to be homologous, differ in their ontogeny, we have good ground for expecting to find evidence that the ontogeny has undergone secondary modification, and that very considerable embryological diversity is quite compatible with close syste-matic affinity.

On the other hand, when two animals whose anatomy does not for-bid comparison exhibit striking ontogenetic resemblances, these must be held to be evidence of phylogenetic relationship.

The strongest evidence of the affinity of Salpa and Pyrosoma is of this sort, and every student who has concerned himself with either the embryology or the asexual multiplication of these animals has expressed or implied his strong conviction of their relationship.

My own studies have forced me to differ from Salensky most essen-tially regarding the part which the follicle cells take in the construction of the salpa embryo, but I fully agree with him (17), p. 84, that the embryological phenomena prove that Salpa and Pyrosoma are closely related. The embryological evidence of their affinity is so fundamental that their whole history must be studied before its weight can be fully appreciated.

I have shown in the chapter on the significance of the Salpa embryo, that the history of its development can be explained only on the hypothesis that it exhibits, in a high degree, secondary complications which are shown at a much more primitive stage by Pyrosoma, which is, so far as our knowledge goes, the only animal which does give us any material aid in the interpretation of the Salpa embryo. I have also shown that there is some reason for believing that the egg of Salpa has at some time in the past been furnished with a food-yolk, and has had a meroblastic mode of development like Pyrosoma, although it may be that these characteristics never became as highly evolved in the ances-tors of Salpa as they are in Pyrosoma.

We must not forget, however, that while there is no other animal known to us with an embryology as much like that of Salpa as Pyrosoma, the differences between the two are very considerable.

The most characteristic peculiarities of the Salpa embryo are its foetal development, and the presence of a placenta and foetal envelopes. The Pyrosoma embryo does not present the slightest trace of these peculiarities, nor is there any indication whatever in the life-history of Salpa of the degeneration which is so remarkably manifested by the Pyrosoma embryo, and we cannot believe that the life-history of either of them is like that of their common ancestor.

In fact the differences are so great that if the resemblance between the embryos were the only thing which these animals have in common, we might well doubt whether this alone is sufficient to prove their affinity, but their whole organization testifies to their very close relationship, and if we make a comparison, not between Salpa and Pyrosoma alone, but between Pyrosoma on the one hand and Salpa, Doliolum and Anchinia on the other, the anatomical resemblance is most impressive. It is exhibited not only by the fundamental plan of their structure, but also in superficial details.

Thus the primary colony in Pyrosoma consists, as it does in Salpa pinnata, Plate I, Fig. 2, of a wheel or rosette made up of a number of zooids placed parallel to each other with their dorsal surfaces outwards, their ventral surfaces towards the axis of the colony, and their mouths all pointing the same way.

It is also interesting to find in both Salpa and Pyrosoma peculiar luminous organs with the same structure, and in positions which, while not strictly homologous, are sufficiently alike to show that they have in all probability been inherited from a common source.

The solitary Salpa pinnata, Plate I, Fig. 6, has five pairs, and the aggregated form of the same species, Plate I, Fig. 3, one pair of longitudinal rod-like organs in the intermuscular spaces at the sides of the dorsal surface.

These organs, which were noted by Förskal, have given rise to much speculation, although no one could examine a living Salpa pinnata without discovering that they are intensely luminous. I am not aware that this interesting fact has ever been recorded. They have been regarded as ovaries (Meyer), or renal organs (H. Muller), but recent writers have refrained from conjectures as to their function.

My first living specimens of this species were examined on deck under the full blaze of the noonday sun, but in spite of the bright daylight my eye was instantly caught by these longitudinal rods which glowed with a light of their own as the animals floated in a dish of

water. In sunlight the luminous organs have a tinge of purple, but at night their light is as white as the glow of an incandescent wire.

Sections show that each organ consists of an aggregation of blood corpuscles in a sinus or dilation of the blood spaces of the body cavity. As most of the corpuscles are swollen, irregular, granular, and in process of degeneration, the sections seem to indicate that the degeneration of the corpuscles is the source of the light, although we cannot look for much information on this point from dead specimens.

Since the time of Savigny, Pyrosoma has been known to have a pair of elongated organs on the sides of the pharynx near the mouth, and as in the case of Salpa, there has been much vague speculation as to their nature.

Savigny thought they were ovaries; Huxley suggested that they were kidneys, and Keferstein and Ehlers believed that they might be organs for the production of buds. According to Herdman (p. 22), Panceri first showed that they are really organs for the production of the light for which Pyrosoma is so famous and from which it gets its name.

Salensky's account of the development of these organs (17), p. 48, and his figure, Taf. 7, Fig. 59, *Lzgr*, seem to show clearly that, in Pyrosoma, as in Salpa, the organs are simply spaces in the body cavity filled with swollen and degenerating cells.

This point of agreement between Salpa pinnata and Pyrosoma, while in itself of little weight, must be regarded as a part of the evidence of their relationship.

Ussow discovered and Bolles Lee has more fully described in the aggregated form of Salpa, a pair of sensory ectodermal tentacles situated near the mouth, and Salensky (17), p. 28, finds in Pyrosoma a pair of tentacles which exhibit a general similarity to those of Salpa, and thus add a little to the weight of the evidence from other sources; although, inasmuch as Salensky's figures and description show that they are at the posterior end of the body in Pyrosoma, we cannot give unqualified assent to his statement that they are identical with the sense tentacles of Salpa in form and structure *as well as position*.

The opinion that Salpa and Pyrosoma are closely related does not, however, rest upon these superficial resemblances, but upon their fundamental identity of structure, although one of the details, the resemblance in their asexual multiplication, is so complete as to be almost enough in itself to establish their affinity.

Kowalevsky's and Huxley's accounts of the proliferous stolon of

Pyrosoma, and the more recent accounts by Seeliger (15) and Salensky (17), would, so far as the anatomical structure of the stolon is concerned, serve as a description of the salpa-stolon. In each case the stolon is bilateral in the same plane as the animal which carries it; in each case it grows out on the ventral middle line near the heart, and in each case it consists of, 1st, an ectodermal tube continuous with the ectoderm of the parent; 2d, an endodermal tube which arises on the ventral side of the pharynx between the folds of the endostyle; 3d, two perithoracic tubes, which, in the primary stolon at least of Pyrosoma (Salensky, 17, p. 475), are ectodermal in ultimate origin, as they are in Salpa; 4th, a nerve tube, or, in the primary stolon of Pyrosoma, a series of nerve vesicles (Salensky, 17, p. 475), which are ectodermal in origin like the nerve tube of Salpa; 5th, two blood tubes, and, 6th, a genital string. The organs which are derived from these various structures are alike in both cases, and my account of the formation of the aggregated salpæ shows that, morphologically, they form a single series placed belly to back, and developing in succession from the tip of the stolon to its base exactly as they do in Pyrosoma, and that each one in the series is joined by its dorsal surface to the ventral surface of the next younger, and by its ventral surface to the dorsal surface of the next older in the series, and that the right and left sides of each one, as well as its dorsal and ventral surfaces, lie, morphologically, in the same relation to space as the corresponding parts of the parent.

In order to appreciate this resemblance in all its details it is necessary to master the minute history in each case, but the phenomena are so complicated and their points of agreement so numerous that the deduction seems to be irresistible that the method of asexual multiplication has been inherited in both Salpa and Pyrosoma from a common source.

I believe, therefore, that the present condition of our knowledge enables us to state with confidence that Pyrosoma, Doliolum and Salpa form a natural group, or great branch from the Tunicate stem.

I have spoken of the various members of this group as *closely related*, but I have not intended to imply that the relationship is in all cases equally close. Anchinia, Dolchinia and Doliolum are clearly more closely related to each other than to any other Tunicate, and the generally accepted view that, in this group, Anchinia is nearest to Pyrosoma seems to have much to commend it. The various species of Salpa are more closely allied to each other than to any other Tunicate, and Salpa

pinnata seems to have more features of resemblance to Pyrosoma than any of the others, but I think this is all we can state with any confidence.

I am quite unable to satisfy myself as to the exact nature of their relationship, or to formulate it either in definitions or in a genealogical tree.

SECTION 5.—*The Nature of the Relationship of Salpa, Doliolum, and Pyrosoma to the Ascidians.*

This subject has been discussed by both Uljanin and Herdman, but there is little harmony in their results, although they agree that Pyrosoma must be regarded as a descendant from the Compound Ascidians.

Uljanin says that we are forced to believe that Doliolum has branched off directly from the Simple Ascidians, since its "ausserordentlich grosse" relationship to them is proved by its embryology. His account of its embryology is very peculiar, however, as he says that the archenteron of the gastrula disappears, and that the entire digestive system of the adult is formed subsequently from an involution of the ectoderm at the oral end of the larva. As nothing of this sort has ever been described in the Simple Ascidians, the embryology of Doliolum can hardly be stated to bear any "unusually great" resemblance to that of the Simple Ascidians.

Uljanin, basing his discussion of the relationships among the Tunicates upon their chordate affinities, and upon the ancestral significance of Appendicularia and the Ascidian tadpole, assumes, as his fundamental principle, that those Tunicates which have most perfectly retained their primitive or ancestral ontogeny, as this is exhibited by primitive chordata, must themselves be most primitive. Even if we admit this, we see that the ontogeny of Doliolum by no means proves its primitive character, for we are forced by his own account to believe that there has been very great secondary modification in its very early stages of development.

The principle cannot be accepted, however, as all embryologists know. No one would hold that a starfish, in which the ontogeny has been simplified by development in brood pouches, is more modern than one with a free larval life. The modified ontogeny is more modern than the unabridged ontogeny, of course, but it by no means follows that the adult animal is also modern.

As the ontogeny of the Simple Ascidians adheres more closely than that of the Compound Ascidians to the primitive chordate type, as this

is exhibited by the anatomy of Appendicularia and by the ontogeny of Amphioxus, he assumes that the simple Ascidians themselves are more primitive than the Compound Ascidians, but his reasoning seems to me to rest upon an erroneous conception of the value and significance of the evidence from embryology.

The resemblance between the larvæ of the Simple Ascidians and the primitive chordata is unquestionably phylogenetic, but it does not by any means follow that the adult animals are older than the Compound Ascidians, and Pyrosoma, Salpa, and Doliolum. The locomotor chordate tadpole larva serves to distribute the species and to establish new sessile animals at a distance from their fixed parents, and these parents might attain to almost any degree of evolution without losing this phylogenetic larval stage so long as its retention continued to be important.

There is no reason for believing that an adult which has retained it is itself primitive, and the great size, complexity and independent individuality of the Simple Ascidians seem to be marks of great specialization rather than of low rank. The most characteristic and dominant organs of a Tunicate are the branchial sac and the atrial system, and these structures are peculiarly complex and highly evolved in the Simple Ascidians.

The leathery, lumpish "sea-squirts" are much less attractive than the delicate, transparent, pelagic Tunicates, whose whole organization is at once seen to be beautifully adapted to the conditions of their life, and it is natural to assume that these latter are the most improved and modern forms, and that the Simple Ascidians are much older, but the muscular mantle and richly ciliated pharynx of the Simple Ascidians are well adapted for protection and nutrition, and the number and diversity of the families and genera and species of Simple Ascidians, and their abundance and wide distribution, prove that their organization has been brought into very complete harmony with their conditions of life, and that, in this respect, they are highly specialized.

The colonial Ascidians spread by budding; and the accidents which break up the colonies scatter the fragments and distribute the species. Small colonies of Perophora and Botryllus are often taken in the tow-net far from land, and the ancestral locomotor stage is much less essential to these animals than to the Simple Ascidians.

The adult Pyrosoma, Doliolum, and Salpa are themselves specialized for locomotion, and as they are not dependent upon local conditions, but are at home all over the ocean, they do not need locomotor larvæ, while

their habit of life seems to be peculiarly favorable for the development of the young upon or within the parent.

While the large food-yolk of Pyrosoma, and the rudimentary condition of its Cyathozooid, and the placenta and fœtal life of Salpa, and the differentiation of a set of Ascidiozoids to carry the sexual animals in Doliolum and Anchinia, are obviously secondary, they do not prove that the adults are any more modern than the Simple Ascidians, nor is the reduction of the tail of the larva to a food reserve anything anomalous, for in all ascidians it is used up as food after it has served its purpose, and we might expect to find it directly devoted to this new use when its locomotor function becomes unnecessary.

It therefore seems to me that the reasons which Uljanin draws from the facts of embryology for holding the view that the Simple Ascidians are more primitive than the other Tunicates, and for regarding Pyrosoma and Salpa as more divergent from the primitive type than Doliolum and the Compound Ascidians, are perfectly consistent with a different interpretation, and we find that Herdman's view is very different.

Briefly summarized, his opinion is that Pyrosoma is not at all closely related to Salpa and Doliolum, and that the two latter forms, with Anchinia and Octacnemius, form a natural group, the Thaliacea, which has been pelagic throughout its whole history, and has nothing in common with the Ascidians except its common descent from an ancestor like Appendicularia, while he regards Pyrosoma as a fixed Compound Ascidian which has secondarily become adapted to a swimming life.

He says (p. 149), "It seems to me that the passage from Appendicularia mossi through Anchinia rubra to Doliolum, and through the ancestral Doliolidæ to Salpa, is so natural and simple that it becomes very improbable that the Thaliacea have ever been fixed forms. It is extremely unlikely that they are, as Uljanin supposes, a group of Simple Ascidians which, after being fixed, betook themselves again to a free swimming mode of life."

On page 124 he says that Appendicularia mossi "is perhaps the nearest form known to the ancestral Tunicates, from which the two great lines of degeneration (?) diverged, the one leading to the Doliolidæ and Salpidæ, and the other to the Simple and Compound Ascidians."

Herdman's introduction of *Appendicularia mossi* into his discussion is unfortunate, and he also fails to give due weight to the evidence of the affinity of Salpa and Pyrosoma which has been pointed out so often by Huxley, Grobben, Salensky, and many others, and in view of all the facts

of the case we must reject the first of his two lines of descent from Appendicularia.

The second line leading to Pyrosoma is perhaps pictured more minutely than the facts warrant, but I think we can safely follow him: 1st, in his statement of the way in which the Ascidian line arose from an ancestor like Appendicularia, and second, in his derivation of Pyrosoma from an Ascidian-like ancestor which is not descended from the Simple Ascidians.

Finally I think we are fully justified in believing with Grobben, in opposition to Uljanin and Herdman, that Salpa (including Octacnemius), Doliolum (including Anchinia and Dolchinia), and Pyrosoma are more closely related to each other than to any other known organism, but I do not see how we can safely commit ourselves to a more minute phylogeny.

Gastrang, in a recent paper on the gill-slits of Tunicates (Proc. Royal Soc. London, May 19, 1892, pp. 505–513), holds that the transverse stigmata of Pyrosoma are *protostigmata;* that they present conditions which are embryonic or larval in the Ascidians, and that all the *secondary stigmata* in a transverse row, in the pharynx of an Ascidian, correspond to or are homologous with a single one of the transverse *protostigmata* of Pyrosoma.

He therefore believes that the pharynx of Pyrosoma approaches nearer to the ancestral type than that of the Ascidians.

The literature of the subject is not all of it in harmony with his view, but I see no valid ground for rejecting his conclusion, which is certainly worthy of a provisional acceptance. Speculations upon the ancestry of Pyrosoma must then conform to the conditions which it imposes, and we cannot derive the pharynx of Pyrosoma from that of any fixed Ascidian which we know.

The facts seem to me to prove that Salpa and Pyrosoma are descended from a fixed form, although Gastrang's studies seem to force us to believe that this fixed form resembled Pyrosoma rather than the Ascidians in the structure of its pharynx, and that it was in this respect more primitive than any Ascidian which we know, although I see no reason why it may not have been the parent of the fixed Ascidians, or why it may not have resembled them in habit of life and in general structure.

In this discussion I have accepted without comment the opinion which seems to be shared by all recent writers on the Tunicata, that

the starting-point for the Tunicate phylum is to be found in a form which was essentially like the modern *Appendicularia*, and that the line which joins the Tunicata to the Vertebrate phylum must pass backwards from the Ascidians through the ancestors of Appendicularia to its point of union with the Vertebrate line.

In this opinion I fully concur, but before I discuss the remote ancestry of the Tunicata, I wish to approach the subject from another point of view, and to speak of the life-history of Salpa in certain broad relations to the question of the origin of the Metazoa. I shall then in another chapter discuss the question of the degeneracy of the Tunicata and the significance of the Appendicularia stage in their development.

CHAPTER VII.

SALPA IN ITS RELATION TO THE EVOLUTION OF LIFE.

Salpa is distinctively a pelagic animal, adapted by its whole structure for a free existence, and for life at the expense of the micro-organisms in the water of the ocean.

To understand its position and significance in the economy of nature, we must have before us the broad outlines, at least, of a picture of the conditions under which oceanic life has been evolved.

I believe that the history of the evolution of Salpa, as told by its embryology, is most suggestive and important, and that it contributes to the solution of some of the most profound and fundamental problems of biology, and brings us into conflict with some of the most favorite dicta of modern morphology. I shall therefore devote considerable space to a review of certain familiar features of ocean life, in order that I may present in this way my view of the significance of the phylogeny of Salpa, in its bearing upon the first principles of morphology.

Contrast between Terrestrial Life and Marine Life.

In a picture of the land, the mind calls up a vast expanse of verdure, broken only by water, and stretching through forest and meadow from high up on the mountains, over hills and valleys and plains, down to the sea.

Our picture of the ocean is an empty waste, stretching on and on with no break in the monotony, except, at long intervals, a floating tuft of sargassum, or a flying fish, or a wandering sea-bird, and we never think of the ocean as the home of vegetable life.

It contains plant-like animals, "zoophytes," in abundance, but while they resemble plants or flowers in form and color, and in their mode of growth, they are true animals and not plants.

At Nassau, in the Bahama Islands, the visitor is taken in a small boat, with windows of plate glass set in the bottom, to visit the "sea-gardens" at the inner end of a channel, through which the pure water from the open sea flows between two coral islands, into the lagoon.

Here the true reef corals grow in quiet water where they may be visited and examined.

The bottom of the boat is below the surface ripples and reflections. When illuminated by the vertical sun of the tropics, and by the light which is reflected back from the white bottom, the pure transparent ocean water is as clear as air, and the smallest object, forty or fifty feet down, is seen distinctly.

As the boat glides over the great mushroom-shaped coral domes which arch up from the depths, the dark grottoes between them, and the caves under their overhanging tops, are lighted up by the sun far down among the flower-animals or anthozoa and the animal plants or zoophytes which are seen through the waving thickets of brown and purple sea fans and sea feathers as they toss before the swell from the ocean.

There are miles of these "sea-gardens" in the lagoons of the Bahamas, and it has been my good fortune to spend many months studying their wonders, but no description can convey any conception of their beauty and luxuriance, and I never spent a day among the reefs without longing, at every turn, for the skill to copy with a brush the new beauties which never ceased to present themselves.

The general effect is very garden-like, and the beautiful fishes of black and golden yellow and iridescent cobalt blue hover like birds among the thickets of yellow and lilac gorgonias. The parrot fishes (Diodon and Ballistes) seem to be cropping the plants like rabbits, but more careful examination shows that they are biting off the tips of the gorgonias and branching madrepores, or hunting for the small crustacea which hide in the thicket, and that all the apparent plants are really animals. The delicate star-like flowers are the vermilion heads of boring annelids, or the scarlet tentacles of actinias, and the thicket is made up of pale lavender bushes of branching madrepores and green and yellow and olive masses of brain coral, of alcyonarians of all shades of yellow and lilac and purple and red, and of red and brown and black sponges. Even the lichens which incrust the rocks are hydroid corals, and the whole sea-garden is a dense jungle of animals where plant life is represented only by a few calcareous algæ, so strange in shape and texture that they are much less plant-like than the true animals.

The scarcity of vegetation becomes still more noticeable when we study the ocean as a whole.

On land, herbivorous animals are always much more abundant and

prolific than the carnivora, as they must be to keep up the supply of food.   Insectivorous birds are very abundant, but they are not numerous enough to keep the plant-eating molluscs and insects in check, and the devastation which is caused every year by the armies of grasshoppers and locusts and herbivorous beetles and by other less conspicuous insects, shows that their natural enemies are not numerous enough to overtax their productive power.

The birds which feed upon grain and seeds and fruit are very abundant indeed, and they sometimes gather at their breeding grounds, or places of assembly, in innumerable multitudes, but the hawks and owls which prey upon them are never numerous.

The small rodents, such as the rats, mice, squirrels and rabbits, are the most abundant and prolific of animals; but the small carnivora are so rare that their very existence is known to few except naturalists and trappers.

The homes of the wild sheep and goats, deer, antelopes, cattle and horses support these large mammalia in incredible numbers, but their carnivorous enemies are never abundant.   It is clear that if the destruction of the plant-eaters exceeded their productive power, both herbivora and carnivora would disappear, and terrestrial life would come to an end.

The animal life of the ocean shows a most remarkable difference, for marine animals are almost exclusively carnivorous.

The birds which live upon the ocean, the terns, gulls, petrels, divers, cormorants, tropic birds and albatrosses, are very numerous indeed; so numerous that in many parts of the ocean some are always visible in calm weather around the vessel, wherever it may be.   The only parallel to the pigeon-roosts and rookeries of the land is found in the dense clouds of sea-birds around their breeding places, but these sea-birds are all carnivorous; most of them are fishers, and others, such as the petrels, scoop up the copepods and pteropods from the surface.   Even the birds of the sea-shore subsist almost exclusively upon animals such as molluscs, crustacea and annelids.

The seals pursue and destroy fishes; the sea-elephants and walruses live upon lamellibranchs; the whales, dolphins and porpoises, and the marine reptiles, all feed upon animals, and most of them are fierce beasts of prey.   The manatee is a vegetable feeder, but it is not strictly a marine animal, since its home is in the mouths of great rivers.

There are a few fishes which pasture in the fringe of seaweed which grows in the littoral zone of the ocean, and there are some which browse

among the floating tufts of algæ upon its surface, but most of them frequent these places in search of the small animals which live among the plants. All the floating fishes whose home is the floating sargassum; the file fishes and trigger fishes (Ballistida); the trunk fishes (Ostracion); the frog fishes (Antennarius); and the puffing fishes (Tetradon and Diodon) are carnivorous, living upon the barnacles and molluscs and hydroids which grow upon the sargassum, or upon the crustacea, young fishes and the floating larvæ which seek its shelter.

In the Chesapeake Bay, the sheepshead (Diplodus probatocephalus) browses among the algæ upon the submerged rocks and piles like a marine sheep, but its food is exclusively animal, and I have lain upon the edge of a wharf watching it crush the barnacles and young oysters until the juices of their bodies streamed out of the angles of its mouth and gathered a host of small fishes to snatch the fragments as they drifted away with the tide.

Many important fishes, like the cod, pasture on the bottom, but their pasturage consists of molluscs and annelids and crustacea, instead of plants.

The vast majority of marine fishes are fierce hunters, pursuing and destroying smaller fishes, and often exhibiting an insatiable love of slaughter, as in the case of our own blue-fish and the tropical albacore and barracuda. Others, such as the herring, feed upon smaller fishes and the pelagic pteropods and copepods; and others, like the shad, upon the minute organisms of the ocean, but all, with few exceptions, are carnivorous.

In the other great groups of marine animals we find some scavengers, some which feed upon micro-organisms, and others which hunt and destroy each other, but there is no group of marine animals which corresponds to the herbivora and rodents and plant-eating birds and insects of the land. The pelagic copepods are, of all the marine Metazoa, the ones whose place in the economy of nature is most like that of the terrestrial plant-eaters. They swarm in innumerable multitudes at the surface of the ocean, and also below it down to a depth of a mile or more, and they furnish the chief food for most young fishes, and for great armies of herrings and pteropods and jelly-fishes and siphonophores, and for most pelagic larvæ.

There are plant-eating molluscs and echinoderms and annelids in the ocean, but not in sufficient numbers to play any conspicuous part in its economy, and the copepods are the only plant-eaters which exist

in sufficient numbers to be compared with those of the land, and the food of the copepods is only partially vegetable, for they devour microscopic animals as well as microscopic plants, and probably to an equal amount.

The group crustacea as a whole is a carnivorous one, however, for while a few subsist on algæ, their number is inconsiderable. Others chew the mud of the bottom and extract its organic matter, but this is chiefly animal and consists of foraminifera and rhizopods and infusoria.

The molluscs as a whole are carnivorous, and while there are many exceptions, such as the nudibranchs for example, many nudibranchs feed on hydroids.

The cephalopods and pteropods and heteropods and many of the gasteropods pursue and destroy their prey, and other gasteropods are scavengers, while the lamellibranchs gather up the microscopic organisms which are drawn into their gills with the water.

The majority of the worms and echinoderms are animal-feeders. Some of them, like the common starfish, are actively predaceous; others, like the crinoids, gather up microscopic organisms from the water; others, such as most holothurians, eat the mud of the bottom and digest out of it the foraminifera and small molluscs and annelids and crustacea which it contains, while others, such as the sea-urchins of the coral reefs, grind away and swallow the living coral. The universal presence of a poisoning apparatus in the cœlenterates shows that the food of this great and important group of marine animals must consist, in the main, of animals which are able to resist or to escape, and observation shows that this is true. Floating jelly-fishes and siphonophores are often found fastened to the half-digested carcasses of sagittas or heteropods or fishes larger than their captors, and they consume enormous numbers of copepods, pteropods, young fish, and pelagic larvæ of all sorts. So far as we know, all the sea-anemones and coral polyps and alcyonarians and hydroids are carnivorous. Some of the discomedusæ, the rhizostomes, feed upon microscopic organisms, but this mode of life is exceptional, and some recent observations, as yet unpublished, by Dr. R. P. Bigelow, show that the food of the rhizostomes consists of copepods.

Except for a few plant-eating fishes and molluscs and worms and echinoderms, all the animals of the ocean fall into two classes, those which subsist on microscopic organisms, and those which prey upon each other and correspond to the rapacious animals of the land.

There is practically nothing in the ocean corresponding to the terrestrial herbivora, and nothing like terrestrial vegetation, except the fringe of seaweeds in the shallow water along the coast, and a few floating islands of algæ like the Sargasso Sea.

While these tracts of vegetation are pretty extensive, they are totally inadequate to support the animal life of the ocean, and as the whole animal world is dependent directly or indirectly upon plants, we must ask what takes the place of terrestrial vegetation.

The Fauna of Mid-ocean.

There is so much room in the vast spaces of the ocean, and the part which is open to our direct observation is such an inconsiderable part of the whole, that it is only when great multitudes of pelagic animals are gathered together at the surface that the abundance of marine life becomes visible and impressive; but some faint conception of the boundless wealth of the ocean may be gained by observing the quickness with which marine animals become crowded at the surface in favorable weather.

On a cruise of more than two weeks from Cape Hatteras to the Bahama Islands I was surrounded continually, night and day, by a vast army of dark-brown jelly-fishes (Linerges mercutia), whose dark color made them very conspicuous in the clear water. They were not densely crowded, although they were so abundant that nearly every bucketful of water we dipped up contained some of them. We could see them at a distance from the vessel, and at noon, when the sun was overhead, we could look down into the water to a great depth through a well in the middle of the vessel where the centerboard hung, and as far down as the eye could penetrate, fifty or sixty feet at least, we could see the brown spots drifting by like motes in the sunbeam. We cruised through them for more than five hundred miles, and we tacked back and forth over a breadth of almost a hundred miles, and they were everywhere in equal abundance.

The recent literature of pelagic exploration, which has been summarized by Haeckel (Plankton Studien : von Ernst Haeckel, Jena, 1890), is full of references to great accumulations of pelagic animals, from which I have selected those which follow.

Chiercha says that during a cruise of forty days between Peru and Hawaii the net brought in from the surface and from all depths down to

about two miles, a multitude of pelagic animals which would be incredible to those who have not witnessed it.

The naturalists of the Challenger found the waters of the equatorial Pacific swarming with life, not at the surface alone, but in its deeper layers, and the ship often sailed through great banks of pelagic animals.

The equatorial Atlantic is like the Pacific, and Chiercha says that its zone of equatorial calms is rich beyond all measure in animal life, and that the water often looks and feels like coagulated jelly.

Of the Indian Ocean, Haeckel says that in his voyage to and from Ceylon he was wonderstruck with the wealth of pelagic life day after day on the mirror-like surface. At night it was an unbroken sheet of sparkling light as far as the eye could reach, and the water which was dipped up at random held such a thick swarm of densely crowded luminous animals (Ostracods, Salpa, Pyrosomas, and Medusæ) that a printed book could be read distinctly in a dark night by this pelagic light.

In temperate and arctic waters there is less diversity, but, as Haeckel shows, there is no evidence of any decrease in individuals, and banks of pteropods (Clio and Limacina), so dense that they seem almost solid, are met even beyond the arctic circle.

Haeckel says that in a cruise to the northwest of Scotland he met with such enormous masses of Limacina that each bucket of water which was dipped up contained thousands.

The tendency to gather in crowds is not restricted to the smaller pelagic animals, and many species of raptorial fishes are found in densely packed banks.

The fishes in a school of mackerel are as numerous as the birds in a flight of wild pigeons. Goode, in his History of Aquatic Animals, tells of one school of mackerel which was estimated to contain a million barrels, and of another which was a windrow of fish half a mile wide and at least twenty miles long; but while the pigeons are plant-eaters, the mackerel are rapacious hunters, pursuing and devouring the herrings, as well as the pteropods and pelagic crustacea.

Herring swarm like locusts, and a herring bank is almost a solid wall. In 1879 three hundred thousand river herring were landed in a single haul of the seine in Albemarle Sound; but the herrings are also carnivorous, each one consuming myriads of copepods every day. In spite of this destruction and the ravages of armies of medusæ and siphonophores and pteropods, the fertility of the copepods is so great that they are abundant in all parts of the ocean, and they are met with in numbers which exceed our powers of comprehension.

On one occasion the Challenger steamed for two days through a dense cloud formed of a single species, and they are found in all latitudes from the arctic regions to the equator, in masses which discolor the water for miles. We know, too, that they are not restricted to the surface, and that the banks of copepods are sometimes a mile thick. When we reflect that thousands would find ample room and food in a pint of water, we can form some faint conception of their universal abundance.

The Primary Food-supply.

As the result of our review, we find that the organisms which are visible without a microscope in the water of the ocean and on the sea bottom are almost universally engaged in devouring each other, and many of them, like the blue-fish and the albacore, are never satisfied with slaughter, but kill from mere sport.

Insatiable rapacity must end in extermination unless there is some unfailing supply, and as we find no visible supply in the water of the ocean we must seek it with a microscope. By its aid we find a wonderfully rich and diversified fauna made up of innumerable larvæ of all sorts of marine animals, together with a few minute and simple metazoa, but these things cannot form the food-supply of the ocean. It is clear that a single carnivorous animal could not exist very long by devouring its own children, and the result must be the same however great the number of individuals or species.

The total amount of these organisms is inconsiderable, however, when compared with the abundance of a few forms of protozoa and protophytes, and both observation and deduction force us to recognize that the most important element in the total amount of marine life consists of some half-a-dozen types of protozoa and unicellular plants, of globigerina and radiolarians, and of trichodesmium, pyrocystis, protococcus, and the coccospheres, rhabdospheres and diatomes.

Modern microscopic research has shown that these simple plants, and the globigerinæ and radiolarians which feed upon them, are so abundant and prolific that they meet all the demands made upon them and supply the food for all the animals of the ocean.

This is the fundamental conception of marine biology. The basis of all the life in the modern ocean is to be sought in the micro-organisms of the surface.

This is not all. The simplicity and abundance of the microscopic

forms and their importance in the economy of nature show that the organic world has gradually shaped itself around and has been controlled by them.

They are not only the fundamental food-supply, but the primeval supply, which has determined the whole course of the evolution of marine life.

The pelagic plant-life of the ocean has retained its primitive simplicity on account of the very favorable character of its environment, and the higher rank of the littoral vegetation and that of the land is the result of hardship.

On the land the mineral elements of plant-food are slowly supplied as the rains dissolve them; limited space brings crowding and competition for this scanty supply; growth is arrested for a great part of each year by drought or cold; the diversity of the earth's surface demands diversity of structure and habit, and the great size and complicated structure of terrestrial plants are adaptations to these conditions of hardship.

The conditions of the surface of the ocean; the abundance and uniform distribution of mineral food in solution; the area which is available for plants; the volume of sunlight and the uniformity of the temperature are all favorable to the growth of plants, and as each plant is bathed on all sides by a nutritive fluid, it is advantageous for the new plant-cells which are formed by cell multiplication to separate from each other as soon as possible in order to expose the whole of their surface to the water. Cell aggregation, the first step towards higher evolution, is therefore disadvantageous to the pelagic plants, and as the environment is so homogeneous at the surface of the ocean that there is little opportunity for an aggregation of cells to gain a compensating advantage by seizing upon a more favorable habitat, the pelagic plants have retained their primitive simplicity.

The list of pelagic micro-organisms is a long one, but a few forms are so predominant that the others have little significance at the present day in comparison, and we may regard the great primary food-supply as made up of two simple protozoa, Globigerina and the Radiolarians, and some five or six unicellular plants.

Of these only two, the Radiolarians and the Diatomes, show any great diversity of species, and while the Radiolarians are so diversified that the Challenger collection alone furnished more than four thousand species, this variety does not obscure the primitive simplicity of the type,

and the most distinctive peculiarity of the microscopic food-supply of the ocean is the very small number of the forms which go to make up the enormous mass of individuals.

The Origin of Pelagic Animals.

All the animals of the ocean are dependent upon the microscopic food-supply, and many of them are adapted for preying upon it directly. Among these Salpa is one of the most conspicuous examples.   It passes its whole life in the open water, and it has no sessile stage in its onto-geny, as many floating animals have.   It abounds in all parts of the ocean, and over some great seas it is always present at the surface.   As the result of three years' observation, Schminkewitch says that the Salpas are perennial pelagic animals, and Chun has shown that they are also found in abundance at great depths.

As long as it is alive and breathing a steady stream of micro-organisms is slipping along its pharynx and down through its œsophagus into its stomach, and sections of the intestine of Salpa afford most beau-tiful preparations of radiolarians and diatomes.

The pelagic food-supply is very ancient, and we have, in Salpa, an animal which has been especially evolved to pass its life swimming through the living broth of the mid-ocean.

If we were to select the typical pelagic animal we should probably choose Salpa, and it is therefore most surprising to find that Salpa itself has not been produced at the surface of the ocean by gradual evolution from a simple pelagic ancestor.

The structure which fits it so well for its mode of life has come to it by the inheritance of peculiarities which were originally acquired by bottom animals in adaptation to the needs of a sessile life.

This is all the more remarkable since both Salpa and its fixed allies show by their embryology that still more remotely they are descended from a pelagic form like Appendicularia.

The place in the pelagic world which Salpa fills so well has been ready for it from primeval times.

Why then has not the simple pelagic Appendicularia given rise, in the open sea, to series of more and more perfected pelagic descendants culminating in Salpa?

Why should the descendants of a pelagic ancestor have passed through a sessile stage before they acquired their improved pelagic structure?

If this were a solitary case it would not deserve notice ; but examination will show that no highly organized animal has ever been evolved at the surface, although all depend on the pelagic food-supply.

The animals which now find their home in the open waters of the ocean are, almost without exception, the descendants of forms which live upon or near the bottom or along the sea-shore or upon the land, and the exceptions are all simple animals of minute size. The metazoa which are primitively pelagic, that is, those which have been pelagic throughout their whole history and do not owe their structure to competition with improved forms from the bottom or the shore, are astonishingly few, and these few are among the smallest and simplest of the metazoa.

It is only necessary to review the chief groups of metazoa in order to perceive that most of their pelagic representatives exhibit the clearest evidence of descent from forms which lived upon or near the bottom or the shore. Many indeed have no pelagic members, but are restricted to the bottom.

The sponges are obviously a bottom group; most of them are fixed, all are sedentary, and their whole organization is an adaptation for life in the bottom.

The coral polyps, actinias and alcyonarias, are among the most characteristic bottom forms, and the abundance of the fossil remains of polyp skeletons proves that these animals became established on the bottom very early, and that the whole history of their evolution has taken place at the bottom. The acraspedote medusæ are universally and justly regarded as the descendants of fixed polyp-like ancestors, and we may state with confidence that they are not primitively pelagic, but that a fixed period in their history has come between the modern swimming jelly-fish and its remote and unknown primitive pelagic ancestor.

The veiled medusæ are usually held to have had a similar history, but I shall soon give my reasons for holding that some of these at least are primitively pelagic. There can, however, be no doubt that the evolution of hydroid cormi has taken place at the bottom. The siphonophores are descended from ancestors like the anthomedusæ, and the various families and genera and species of siphonophores have most certainly been produced by divergent specialization among pelagic forms, and the greater part of their history, if not the whole of it, is therefore pelagic.

The echinoderms are most characteristic inhabitants of the bottom, as they have been from palæozoic times, and while synapta is sometimes

found at the surface of the open ocean, this is exceptional, and we may state without hesitation that the evolution of the echinoderms has taken place at the bottom. This is equally true of the brachiopods and of most of the animals classed as vermes, the gephyreans, bryozoa, nemertians, and so forth. The pelagic annelids, such as Tomopteris, are secondary modifications of bottom forms, and while some of the more primitive annelids may possibly be originally pelagic, the group as a whole is as characteristic of the bottom as the echinoderms.

Many groups of crustacea have pelagic representatives, and the pelagic crustacean fauna is rich and varied, but in most cases the pelagic forms show unmistakable evidence of secondary change of habit, and all the higher crustacea have been evolved at the bottom in adaptation to a bottom life.

I shall soon give my reasons for believing that there is one important exception to this rule, however, and I shall try to show that there is good ground for holding that the copepods are primitively pelagic, and that while the greater part of the history of the crustacea is bottom history, the characteristics of the crustacean type were outlined in pelagic animals at a very early period in the history of the metazoa.

The heavy calcareous shells of the mollusca could not have been acquired at the surface, and that most characteristic molluscan organ, the lingual ribbon, is adapted for attacking more solid bodies than the delicate primitive pelagic animals. The classes and orders of mollusca must have been evolved at the bottom, and there is ample evidence that the swimming shelless gasteropods and cephalopods have, like those great pelagic groups the pteropods and heteropods, been secondarily adapted for a pelagic life.

Many of the marine fishes are strictly pelagic, and the structure and habits of fishes are in all respects so well fitted for a wandering life in the open water that the pelagic habit of fishes seems at first sight to be their most distinctive peculiarity, although a little examination will show that there is ample evidence that it is secondary, and not primitive.

The perfection of their adjustment to a free life in the open sea is no evidence that this life is primitive, for the highest marine animals and those whose adaptation to a pelagic life is most complete, the sea-birds and cetacea and marine reptiles, are air-breathing terrestrial animals which have gone back into the ocean.

The most primitive groups of living fishes are the cyclostomes, elasmobranchs and ganoids. The cyclostomes are too small a group, and

the living forms are too aberrant in habit, to contribute much information regarding the nature of the primitive vertebrates, but they exhibit no evidence of adaptation to a pelagic life, and our scanty knowledge of them is quite in harmony with the view that their remote ancestors were bottom animals.

The case is very different as regards the great groups of modern fishes for which the term palæichthyes is often used; the sharks, rays and ganoids.

The living representatives of these great and ancient groups are of peculiar interest to naturalists on account of their close affinity to the oldest vertebrate fossils which have been discovered. These points of resemblance to the more modern, but still ancient, amphibia and teleosts show that the modern palæichthyes have preserved their ancient structure with very slight modification, and that we have in them one of the most remarkable stem forms in the whole animal kingdom. This is shown still more conclusively by the fact that some of the palæozoic families of elasmobranchs have lived through period after period of geological history and have held their ground up to our own times.

The abundance and variety of the remains of elasmobranchs in the palæozoic rocks prove the great development of the group at this remote and early period, and the silurian sharks probably differed but little from those of the present day, although we are forced to see in them the ancestors of the ganoids and of all the divergent groups of extinct and living vertebrates.

Of the three groups of modern elasmobranchs, two, the chimæras and the rays, are bottom-feeders. The whole organization of the ray is as obviously adapted for life upon or near the bottom as that of a bird is for life in the air, and the flat pavement teeth are adapted for crushing and grinding the hard-shelled molluscs and crustacea and echinoderms of the bottom.

It is true that the sawfish is not confined to the bottom, and the devil-fishes often capture their prey at the surface. In the West Indies they are often found very far from land, but these cases are exceptional, and the true rays rarely leave the bottom, nor are they adapted for rapid movement through the water.

The rays are undoubtedly much more modern than the true sharks, but there is ample evidence that they have retained habits of life which are common to all the primitive elasmobranchs.

Many of the modern sharks live on or near the bottom, where they

are found in immense numbers and at considerable depths. In 1888 I was invited by Marshall McDonald, the Superintendent of the U. S. Fish Commission, to make use of the opportunity for surface collecting which was afforded by an expedition which was sent out to fish with hook and line on the bottom and along the edge of the Gulf Stream. The fishing commenced at the 500 fathom line, and every time the line was taken in we found numbers of dogfish (Scyllium) on the hooks, even when the water was considerably more than half a mile deep.

Many genera of sharks, such as the houndfish (Mustelus) and the dogfish (Scyllium), are known to feed upon the molluscs and crustacea and worms of the bottom, and the flat pavement-teeth of other genera whose habits are less known show that their mode of life is the same. Some of the bottom-feeding sharks (Cestracion for example) are the oldest of living vertebrates.

The mailed ganoids were undoubtedly derived from a shark-like ancestor, and the structure of the oldest ones, such as perichthys, coccosteus and cephalaspis, shows that they were not very rapid swimmers. They were, undoubtedly, bottom-feeders like the modern sturgeon, and like many large and important families of modern teleosts, such as the cod, the siluroids and the pleuronectidæ.

So far as we know the palæozoic waters from fossils, there were no active locomotor animals of large size to furnish prey for raptorial fishes, and the existence at the present day of so many species and genera and families of bottom-feeders, and the fact that the most archaic forms have this habit, are all grounds for believing that the fishes are secondarily adapted to a pelagic life, like the sea-birds and the cetacea.

So far as amphioxus furnishes evidence, this bears in the same direction, for its home is in the sand of the bottom. In fact it may almost be called a subterranean animal, for when it is placed in an aquarium it sinks into the sand at the bottom and disappears at once, and it makes its way through the sand with great ease and rapidity.

All the evidence shows that the primitive vertebrates lived upon or near the bottom, and that the early steps in the evolution of the classes of vertebrated animals were made at the bottom.

As the result of this review we see that the evidence from palæontology, from embryology, and from the structure and habits of living animals all bears in the same direction, and shows that there are no large or highly organized animals which have been pelagic through all the stages of their evolution, and that, in this particular, the life-history of Salpa is not exceptional, but typical.

In its descent from an inhabitant of the bottom and in its secondary adaptation to a pelagic life, its history resembles that of all the highly organized pelagic animals.

Embryology also gives us good ground for believing that Salpa follows the analogy of all the metazoa in its still more remote descent from a small and simple pelagic ancestor, and there is good ground for believing that the earliest metazoa were all pelagic, and that they were represented at a very early period in the history of life by floating or swimming animals of minute size and simple structure. We may see in the free larval forms of many marine metazoa, such as the tornaria of balanoglossus, the swimming echinoderm larva, the ascidian tadpole, the floating ciliated larvæ of annelids, brachiopods and molluscs, in the cœlenterate planula, and, as I believe, in the crustacean nauplius, traces of this primitive mode of life; often obscured or complicated by more recent adaptation and sometimes almost obliterated by secondary changes.

When this fact is seen in all its bearings and its full significance is grasped, it is certainly one of the most noteworthy and instructive features of the history of evolution.

The food-supply of the ocean consists of a few species of unicellular microscopic plants, and of a few simple protozoa which feed upon them. This supply is inexhaustible, and it is the only source of food for all the inhabitants of the ocean, except a few which live upon floating sargassum and the littoral algæ, and the drainage from the land.

Many marine animals are adapted for direct subsistence upon these organisms, and some of them, like Salpa, are universally distributed and are found in enormous numbers in all parts of the ocean.

The food-supply is not only inexhaustible, it is also primeval, and all the life of the ocean has gradually taken shape in direct dependence upon it during the history of its evolution.

In view of all these facts we cannot but be profoundly impressed by the thought that all the highly organized marine animals are products of the bottom, or of the shore, or of the land, and that while the largest animals on earth are pelagic, the few which are primitively pelagic are very small and very simple.

The reason is obvious. The conditions of pelagic life are so easy that there is no fierce competition, and the inorganic environment is so simple that there is little chance for diversity of habits.

The growth of terrestrial plants is limited by the scarcity of food,

but there is no such limit to the growth of pelagic plants or the animals which feed upon them, and while the balance of life is undoubtedly adjusted, competition for food is never very fierce even at the present day, when the ocean swarms with highly organized animals which have become secondarily adapted for a pelagic life. Even now the destruction or escape of a microscopic pelagic organism depends upon the accidental proximity or remoteness of an enemy rather than upon defense or protection, and survival is determined by space relations rather than by a struggle for existence.

The abundance of food is shown by the ease with which wanderers from the land, like birds, find places for themselves in the ocean, and the rapidity with which they spread over its whole extent.

As a marine animal, the insect, halobates, must be very modern as compared with most pelagic forms, yet it has spread over all tropical and subtropical seas, and it may always be found skimming over the surface of the water as much at home as a gerris in a pond. I never found it absent in the Gulf Stream when conditions were favorable for collecting.

The easy character of pelagic life is also shown by the fact that the larvæ of innumerable animals from the bottom and the shore have retained their pelagic habit, and I shall soon refer to facts which prove that the larva of a shore animal is safer at sea than it is near the shore. The absence of fierce competition in the open ocean is well shown by the simultaneous existence in the modern ocean of graded stages in the evolution of a type, such as the series of Pelagidæ; and also by the persistency of a stem form like the elasmobranch, side by side with, and often in competition with, various improved lines of divergent descendants.

In the primitive pelagic fauna and flora there was little opportunity for an organism to gain superiority by seizing upon an advantageous site or by acquiring peculiar habits, for one place was like another, and peculiar habits could count for little in comparison with accidental space relations.

After the pelagic fauna had been enriched by the addition of all the marine animals which are secondarily pelagic, competition with these improved forms from the bottom brought about improvements in those which were strictly pelagic in their origin, and through this competition, complicated animals of considerable size, like the siphonophores, have been evolved at the surface, but while their whole history has thus been pelagic they are not primitively pelagic; that is, they are not the out-

come of purely pelagic influence. The wanderers from the bottom have introduced another factor in the evolution of pelagic life, for their bodies have been utilized for purposes of protection or concealment or on account of other advantages, and we now have fishes which shelter themselves in the poisoned curtain of physalia; crustacea which live in the pharynx of salpa; barnacles and sucking fishes fastened to whales and turtles, besides a host of external and internal parasites. The primitive ocean furnished no such opportunity, and the conditions of pelagic life must, at first, have been extremely simple.

Among the higher metazoa and the higher plants size is, in itself, an important factor in evolution. Variations in the constituent cells of a large organism are continually being seized upon and fixed by natural selection, on account of their value in the functions of relation to other parts. Primitive pelagic organisms are all minute, and it is easy to understand why. To plants which are bathed on all sides by food, like the pelagic protophytes, small size is advantageous, since a small body has a larger surface in proportion to its bulk than a large one; and the pelagic plants are, as I have shown, most favorably placed for rapid growth when new cells separate as soon as they are formed, and thus expose all their surface.

The same ratio between bulk and nutritive surface tends to limit in the same way, if not to the same degree, the growth of the pelagic animals which live in the midst of an abundant supply of vegetable food.

Competition was not entirely absent among the primitive pelagic organisms, for the conditions of life are never absolutely uniform, although the possibilities of evolution must have been extremely limited and the progress of divergent modification very slow, so long as life was restricted to the waters of the ocean.

There can be no doubt that pelagic lfe was abundant for a long period during which the bottom was uninhabited. The history of the slow process of geological change by which the earth gradually assumed its present character, presents a boundless field for speculation, but there can be no doubt that the surface of the primeval ocean became fit for life long before the deeper waters or the sea-floor.

The early steps in the evolution of plants must have been taken in the transparent surface water under the influence of sunlight, and as both animals and plants are dependent upon oxygen, the primal flora and fauna must have lived in aerated water. The oxygen which is

diffused through the ocean from the surface, where it is absorbed from the air, is gradually exhausted by oxidizable substance, both inorganic and organic, and it diminishes with the distance from the source of supply at the surface. The oceanic circulation tends to equalize its distribution, and no part of the ocean now seems to be totally without oxygen. Oxygen has been shown to be reduced to a minimum at the bottom of some of the great depressions of the sea-floor, and it is clear that a slight change in the conditions which influence it might render the sea-bottom unfit for life.

In early palæozoic times the sea-floor was perhaps more level than it is now, and there may have been no deep hollows like those in which the oxygen is now found to be deficient, but the average depth must have been considerably greater, when all the water which is now locked up in the sedimentary rocks of the bottom and of the shores was still free in the ocean. The circulation may also have been less active when geographical conditions were more simple, and the air was undoubtedly less rich in oxygen in early palæozoic times than it is at present.

It is therefore easy to understand that long after the crust of the earth had acquired essentially its present character, there may have been a period when the supply of oxygen was so scanty that the activities of pelagic organisms and the products of their decomposition used it up in the surface water, so that life on the bottom was impossible at a time when the superficial water supported a luxuriant fauna and flora.

During this period the proper conditions for the production of large and complicated organisms did not exist, and while the total volume of life was probably very great, it consisted of the organisms of minute size and simple structure which I have termed the primitive pelagic fauna and flora.

The Primitive Pelagic Fauna.

In using this term I do not, of course, intend to imply that these organisms are the beginning of life, or to express any opinion as to the way in which life first came into existence. I use it merely as a convenient designation for the total sum of the organisms which have been evolved by purely pelagic influences from a starting-point which is absolutely unknown at present.

The attempt to reconstruct in imagination the primitive pelagic fauna and flora is most fascinating, but all the available evidence is indirect, and as we can have little hope of finding any record of it in the rocks, we must trust to deduction rather than observation.

The modern pelagic protophytes have probably retained nearly their ancient form, but the modern radiolarians and pelagic foraminifera exhibit indications of secondary adaptation, and they have undoubtedly been modified by competition with improved organisms from the bottom.

All the metazoa have pelagic larvæ, or else larval or embryonic stages, which must be regarded as the degenerated vestiges of a pelagic habit; but in most cases these larvæ have been so much changed by the accelerated development of adult features, or by the acquisition of habits or structures to fit them for the conditions of modern pelagic life, that we can deduce little more from them than the former existence of pelagic ancestors. When a pelagic larva is still represented by a modern pelagic adult of minute size and simple structure, as the tadpole larva of ascidians is represented by Appendicularia, we may be confident that it is a pelagic production, and that it existed in the primitive pelagic fauna, although this view is directly opposed to accepted dogmas regarding the origin of the Chordata. When all the members of a great group have a definite pelagic larval stage which adheres to the same plan of structure in all of them, we may be pretty confident that this larva is the representative of a primitive pelagic adult animal, even if this ancestor has now no unmodified descendants.

To my mind the best example of the retention, by all the members of a great group, of a larval stage which represents an extinct primitively pelagic ancestor is to be found in the crustacea nauplius, and notwithstanding the popular verdict against it, I do not hesitate to regard the nauplius as a pure pelagic product, and to include it in the primitive pelagic fauna, although I shall discuss this question further on. In cases like that of the echinoderms, where the pelagic larvæ of the various classes and orders are very different from each other in the details of their organization, we are hardly safe in assuming more than the primitive existence of an unknown pelagic organism, from which they have been derived. This is true to even a greater degree of the trochic larvæ of annelids, molluscs, etc., but while there is little ground for regarding the forms of these modern larvæ as ancestral, we must regard their pelagic habit as an inheritance from unknown ancestors in the primitive pelagic fauna, in which we must therefore include representatives of such larvæ as the molluscan veliger, the nemertian pilidium, the actinotrocha of phoronis, the brachiopod larva, the cœlenterate planula, and so forth, although we are quite unable to say how many independent starting-points these various metozoan lines had in the primitive pelagic

fauna, or what these starting-points were like. Our inability to describe or picture these ancestral forms is no reason for doubting their reality, for in biology the weight and certainty of a deduction are often independent of its definiteness. We may, for example, feel sure that the cetacea are descended from terrestrial animals and yet find it impossible to picture their ancestor, or even to decide whether their ancestral lines converge into one stem before or after the pelagic habit was acquired.

We may in the same way feel sure, even in the absence of sufficient evidence to trace their direct paths, that all the great groups of metazoa ran back to minute pelagic ancestors, and we must, therefore, include in the primitive pelagic fauna a great, but indefinite, number of distinct and somewhat widely separated ancestral forms, and together with them, no doubt, an equal or greater number of somewhat similar forms which have been exterminated and have left no descendants. In these extinct forms we should, if we could study them, find the connecting links between divergent groups, and we would thus be able to complete the genealogical tree of the metazoa by bringing together the great divergent branches of the metazoan stem whose primary relationships now seem beyond discovery.

In addition to the primitive pelagic animals which are known to us only by the traces of their former existence which they have left in the structure and habits of modern larvæ and embryos, there are a few modern pelagic adult animals which show by their minute size and simple structure and by their systematic affinities that they are primitive pelagic animals, owing their structure to purely pelagic influences.

Appendicularia is a good example of this class, and I believe that the copepods are the most important group of the primitively pelagic metazoa.

The Origin of the Crustacea.

The view that the copepods are degenerated descendants from crustacea like the phyllopods, and that the crustacea were evolved on the bottom, and that the pelagic habit of the copepods is secondary, is so generally accepted that it is hardly worth while to advance a different view in this place where there is no room for its exhaustive treatment.

The consideration which seems to have the greatest weight with morphologists is the supposed necessity of a phylogenetic explanation of metamerism, but a little reflection will show the persistent existence of an influence which tends to metamerism at the present day.

For this influence, which is shown by such phenomena as the inheritance by the child of polydactylous feet from the polydactylous hands of the parent, or the development of wing-feathers on the toes of fantail pigeons; the influence which has carried the feet of the horse family along the same line of evolution with the hands, I have, in another place, proposed the term ontogenetic inheritance. Among the arthropods, examples of this sort of modern metamerization are very common, both as normal features of their structure, in the movable body-rings of the ocular and antennary somites of stomatopods, for example, and as monstrosities, as in the twelve-legged coleoptera.

I believe that a thorough study of this most interesting and instructive class of facts will convince any one that there is no philosophical necessity for assuming that the primitive crustacean had a highly metamerized body like that of a phyllopod, and that all the common features in the structure of arthropods may have been derived from a common ancestor as simple as a nauplius.

The analogy between the parapodia of annelids and the limbs of crustacea has been held to prove that the primitive crustacean limb was not a rowing organ fitted for a pelagic life, like the limbs of the nauplius and the copepod, but flat and leaf-like and adapted for movement over the bottom.

It is hardly possible, however, to believe that the arthropods have been derived from the higher polychætous annelids, and as the simpler and more primitive annelids have no parapodia, the resemblance, which is not actually very noteworthy, can be nothing more than an analogy.

There are plenty of degenerated copepods, and we have in their structure abundant proof of the degeneracy, and an adequate explanation of it in their parasitic habits, but they are degenerated descendants of ordinary swimming copepods, and not of phyllopods, and there is no reason for holding that the copepodan type itself is degenerate, except the supposed exigencies of morphological philosophy.

The active locomotor habits of the eucopepods of the open ocean would seem to be conducive to advancement rather than to degeneration, and the occurrence of phyllopods in the lower Cambrian is, of course, no more evidence that they are primitive crustacea than the occurrence of pteropods and gasteropods is that they are primitive molluscs.

I am unable to see any valid objection to the view that the copepods are primitively pelagic; that they have been evolved at the surface of

the ocean from pelagic nauplii, and that the great group crustacea has been derived from them.

We have already seen that the eucopepods are the chief intermediary between the micro-organisms of the ocean and the larger and higher marine animals; that they prey upon the protophytes and protozoa, and in their turn supply either directly or indirectly most of the food for the large inhabitants of the water; that most pelagic larvæ feed upon them; that they are the food of the great pelagic banks of pteropods and heteropods, of many cœlenterates, of the young of most fishes, and of some of the most abundant and important adult fishes, like the herring, and that the sea-birds, the cetacea, and in fact almost all of the larger pelagic animals, prey upon animals which in their turn prey upon copepods.

The animals which are most important at one period in the earth's history are often replaced by others at another period, and it is, of course, possible that the modern copepods now fill a place which was in former times filled by something else; but as their organization, as compared with that of the other crustacea, exhibits all the characteristics of a primitive pelagic stem-form, and inasmuch as the remains of animals, like the pteropods, which now live almost entirely upon copepods, are found in the oldest fossiliferous rocks, there is every reason to believe that the group formed an important constituent of the primitive pelagic fauna.

No one who advocates at our time the morphological heresies which are involved in the view that appendicularia is a stem-form which is pelagic in its origin; that the nauplius is a persistent representative of the primitive crustacea, and that the whole history of the copepods has been pelagic; and that the veiled medusæ have been evolved in direct relation to pelagic influences; no one who makes these statements can hope to escape the charge that his view "ist die unwahrscheinlichste von unwahrscheinlichkeiten."

The books all tell us that the free active appendicularia is the "degenerated" descendant of an ancestor which crept over the bottom; that the nauplius is a secondary larval form; that the active free-swimming copepods are degenerated phyllopods; and that the locomotor hydro-medusa is, in its origin, a specialized member of a sessile, polymorphic, hydroid cormus.

The first of these opinions, that appendicularia is a degenerated form, rests upon a supposed necessity for deriving the body of a vertebrate, which consists of a series of segments homologous with each other,

but highly differentiated among themselves, from an ancestral aggregation of similar, but less differentiated, segments.

The second opinion, that the copepods are degenerated and that the nauplius is a secondary larval form, is the result of a supposed necessity for explaining the segmentation of the arthropods in the same way, while the third view has its origin in the belief that the polymorphic members of a hydroid cormus must have arisen through specialization and division of labor from an ancestral undifferentiated aggregation.

These are a few, from among many, illustrations of the general acceptance among morphologists of a dogma which, while it is often refined and qualified until its character is almost lost, may be broadly stated as a belief that the homology between different parts of the same organism is always to be explained, like the homology between corresponding parts of different animals, as the result of phylogenetic inheritance; or, to state it in a different way, that the vegetative duplication of parts in animals has a phylogenetic significance, and implies descent from a duplicated ancestor.

The dogma is not the dictum of any one teacher, and it has grown almost imperceptibly from its starting-point in the discovery that the body of a metazoon is an aggregation of cells, each with an individuality of its own, specialized and differentiated by polymorphism and division of labor, and each one homologous with an unicellular organism.

The dogma has been a most useful and suggestive working hypothesis when well controlled, but when uncontrolled it has led to the most fantastic and grotesque unscientific speculation. The climax of inconsistency into which its blind adherents have been led was well shown by the simultaneous appearance, in a recent morphological journal, of two memoirs, one an essay on "The Origin of the Vertebrates from the Arachnids," and the other on "The Origin of Vertebrates from a Crustacean-like Ancestor."

After my first examination of the second of these memoirs I laid it down, much distressed in mind by the thought that this author had unkindly descended from the sphere of experimental research in physiology, to expose the unscientific methods of the morphologists by a severe and well merited, if somewhat ponderous, satire.

In my next chapter on the morphological significance of appendicularia I shall try to show that there is no philosophical necessity for a phylogenetic explanation of duplicated structures in animals, whether they are radial, bilateral, metameric or indefinite, and I must refer the

reader to that chapter for my reasons for including appendicularia, the copepods and the veiled medusæ among the primitive pelagic animals.

The Phylogeny of the Metazoa.

The primitive pelagic fauna, before the influence of the bottom and of the shore had been brought to bear upon it, consisted of small animals of simple structure; but we are forced, by the facts of comparative anatomy and embryology, to believe that a number of distinct types of structure were found among them.

Most of the great metazoic stems show by their embryology that they run back to simple and minute pelagic ancestors, and that their common meeting-point must be projected back to a still more remote time, before the differentiation of their pelagic ancestors had been effected. After we have traced each great line of metazoa as far back as we can from the study of fossils and by the aid of comparative morphology, we still find these lines distinctly laid down. The lower Cambrian crustacea, for example, are as distinct from the lower Cambrian echinoderms or pteropods or brachiopods or lamellibranchs, as they are from those of the present day. The efforts of anatomists and embryologists to reconstruct the primary phylogeny of the metazoa have so far yielded few trustworthy results, and the results which are most trustworthy are usually those which are the most indefinite.

We are therefore forced to believe that the early steps in the establishment of the various types of metazoa were taken under conditions which had some essential difference from those which have prevailed, without any fundamental changes, from the time of the oldest fossil to the present day; and we are also forced to believe that most of the great lines of descent were represented at some time in the remote past by ancestors which, living a different sort of life, differed essentially in structure as well as habits, from the representatives of the same types which are known to us. Furthermore, embryology teaches that each great group still bears internal evidence of descent from pelagic ancestors, and while the characteristics of these ancestors are in most cases unknown, a few, like appendicularia, are still found alive.

Our knowledge of the evolution of the metazoic types has certain general features which are essentially the same for all, but each group has also in its history much that is individual, and any general statement requires so much qualification that the history of an illustrative group is more instructive than a general summary.

In the echinoderms we have a well-defined type represented by abundant fossils, very rich in living forms, very diversified in its modifications, and therefore well fitted for use as an illustration.

This great stem contains many classes and orders, all constructed on the same plan, which is sharply isolated and quite unlike the plan of structure in any other group of animals. All through the series of fossiliferous rocks echinoderms are found, and the plan of structure is always the same. Palæontology gives us most valuable evidence regarding the course of evolution within the limits of a class as in the crinoids and in the echinoids; but we appeal to it in vain for light upon the organization of the primitive echinoderm, or for connecting links between the classes. To our questions on these subjects and on the relation of the echinoderms to other animals, palæontology is silent, and throws them back upon us as unsolved riddles.

The morphologist unhesitatingly projects his imagination, held in check only by the laws of scientific thought, into the dark period before the times of the oldest fossils, and feels absolutely certain of the past existence of a stem form, from which the classes of echinoderms have inherited the fundamental plan of their structure, and he affirms with equal confidence that the structural changes which have separated this ancient type from the classes which we know were very much more profound and extensive than all the changes which each class has undergone from the earliest palæozoic times to the present day.

He is also disposed to assume, but, as I shall show, with much less reason, that the amount of change which structure has undergone is an index to the length of time which the change has required, and that the period which is covered by the fossiliferous rocks is only an inconsiderable part of that which has been consumed in the evolution of the echinoderms.

The morphologist does not check the flight of his scientific imagination here, however, for he trusts implicitly to the embryological evidence which teaches him that, still further back in the past, all the echinoderms were represented by a minute pelagic animal which was not an echinoderm at all in any sense except the ancestral one, although it was distinguished by features which natural selection has converted, under the influence of more modern conditions, into the structure of echinoderms. He finds, in the embryology of modern echinoderms, phenomena which can bear no interpretation but this, and he unhesitatingly assumes that they are an inheritance which has been handed down from genera-

tion to generation through all the ages from the prehistoric times of zoology.

Other groups tell the same story with equal clearness. Who can look at a living lingula without being overwhelmed by the effort to grasp its immeasurable antiquity, and by the thought that, while it has passed through all the chances and changes of geological history, the structure which fitted it for life on the earliest palæozoic bottom is still adapted for a life in the sands of the modern sea-floor?

The everlasting hills are the type of venerable antiquity; but lingula has seen the continents grow up, and has maintained its integrity unmoved by the convulsions of nature which have given to the crust of the earth its present form.

As measured by the time-standards of the morphologist, lingula itself is modern, for its life-history still holds, locked up within it, the record of a structure and of a habit of life which were lost in the unknown past at the time of the lower Cambrian, and it tells us, vaguely but unmistakably, of a life at the surface of the primitive ocean at a time when the brachiopod stem was represented by minute and simple pelagic animals.

Broadly stated, the history of each great line of metazoa has been like that of the echinoderms or brachiopods, for while the brachiopods are certainly much more closely related to the polyzoa or the gephyreans than to the echinoderms, and while these latter are nearer to the chordata than to the brachiopods, yet each great line stands sharply by itself.

The oldest pteropod or lamellibranch or crustacean or echinoderm or vertebrate which we know from fossils exhibits its own type of structure with perfect distinctness, and later influences have done no more than to expand and diversify the type, while anatomy fails to guide us back to the point where these various lines met each other in a common source, although it forces us to believe that this common source once had an individual existence.

Embryology teaches that each line once had its own pelagic representatives, and that the early stages in the evolution of each type have passed away and left no record.

The palæontological side of the subject has recently been ably summed up by Walcott in an interesting memoir on the oldest fauna which is known to us from fossils (The Fauna of the Lower Cambrian or Olenellus Zone, by Charles Doolittle Walcott, U. S. Geological Survey, 10th Annual Report, Washington, 1890).

The fossils of the lower Cambrian are not absolutely the oldest known, but it is the oldest fauna which is represented with sufficient completeness for a general view, and is, therefore, interesting to biologists.

Walcott says that no plants are known in the rocks of the lower Cambrian, and that he has satisfied himself, after a study of all the reputed species of algæ, that they are not plants, but the trails of worms or molluscs.

The number of species is small, but their diversity is most noteworthy and remarkable.

Walcott's collection of 141 American species from the lower Cambrian is distributed over most of the marine groups of the animal kingdom, and, except for the absence of the remains of vertebrates, the whole province of animal life is almost as completely covered by these 141 species as it could be by a collection from the bottom of the modern ocean.

Four of the American species are sponges, two are hydrozoa, nine are actinozoa, one an echinoderm, twenty-nine are brachiopods, three are lamellibranchs, thirteen are gasteropods, fifteen are pteropods, eight are crustacea, fifty-one are trilobites, and the trails and burrows show the existence of at least six species of bottom forms, probably worms or crustacea.

The most noteworthy characteristic is the completeness with which these new species outline the whole fauna of the modern sea-floor.

Nothing brings home more vividly to the zoologist a picture of the diversity of the lower Cambrian fauna and of its intimate relation to the bottom fauna of to-day than the thought that he would have found, on the old Cambrian shore, about the same opportunity to study the embryology and anatomy of pteropods, gasteropods and lamellibranchs and crustacea and medusæ that he now has at a marine laboratory, and that his studies in phylogeny would have had about the same form then that they have now.

Biological evidence based on embryology and anatomy and on the habits and affinities of animals is justly regarded, by zoologists at least, as a more perfect record of the early history of life than palæontology, and we accept, without question, proofs of phylogeny which refer to a time very much more remote than the age of the oldest fossils.

We must not forget, however, that our generalizations in primitive phylogeny rest for the most part on the study of swimming or floating larvæ of minute size and simple structure, which we can have little hope of finding as fossils.

In the formations which follow the lower Cambrian, species gradually become more numerous, but this is due to divergent specialization, and Walcott says that if a comparison be made between the Olenellus Zone (lower Cambrian) and the Silurian fauna, the superiority of the latter in number of species, genera and families is at once apparent.

"If the comparison be extended to class characters, the disparity between the two is very much reduced, and it is made evident that the evolution of life between the epoch of the Olenellus fauna and the epoch of the Ordvician fauna has been, with one or two exceptions, in the direction of differentiating the class types that existed in the earlier fauna."

The ground which we have covered in our review of these various broad aspects of the animal kingdom brings us, then, to the following point of view:

There are no highly organized animals which have been pelagic through all the stages of their evolution. The metazoa, which have been pelagic through their whole history, are either small and simply organized, as compared with the higher representatives of the group to which they belong, like appendicularia, or else, like the siphonophores, they have been perfected through competition with higher types.

Marine life is older than terrestrial life, and as all marine life has shaped itself in relation to the pelagic food-supply, this itself is the only form of life which is independent, and it must therefore be the oldest. There must have been a long period in primeval times during which there was a pelagic flora and fauna, rich beyond limit in individuals, but made up of only a few small simple types. During this time the pelagic ancestors of all the great groups of metazoa were slowly evolved, as well as others which have no living descendants. So long as life was restricted to the surface, no great or rapid advancement through the influences which now modify species was possible, and we know of no other influence which might have replaced these. We are, therefore, forced to believe that the differentiation and improvement of the primitive flora and fauna was slow, and that for a vast period of time life consisted of an innumerable multitude of pelagic organisms made up of a few forms. During the time which it took to form the thick beds of older sedimentary rocks the physical conditions of the ocean gradually took their present form, and during a part, at least, of this period, the total amount of life in the ocean may have been about as great as it is now without leaving any permanent record of its existence, for no rapid

advancement took place until the advantages of a life on the bottom were discovered.

The Discovery of the Bottom, and its Effect on Evolution.

We must not think of the populating of the bottom as a physical problem, but as colonization, very much like the colonization of oceanic islands. Physical conditions for a long time made it impossible, but its initiation was the result of biological influences, and there is no reason why the starting-point should be the point where the physical obstacles were first removed. It is useless to speculate upon the character of the physical obstacles; there is reason to believe that one of them, probably a very important one, was the deficiency of oxygen in deep water.

Whatever their character may have been they were all, no doubt, of such a nature that they first disappeared in the most shallow water around the coast, but it is not probable that bottom life was first established in shallow water, or before the physical conditions had become favorable at considerable depths.

The sediment near the shore is destructive to most pelagic animals, and recent explorations have shown that a stratum of water of very great thickness is necessary for the complete development of the pelagic flora and fauna. It is a mistake to picture pelagic life as confined to a thin surface stratum. Pelagic plants probably flourish as far down as the light penetrates, and pelagic animals are abundant at very great depths. As the earliest bottom animals must have depended directly upon the floating organisms for food, it is not probable that they first established themselves in shallow water, where the food-supply is not only scanty in amount but also mixed with sediment; nor is it probable that their establishment on the bottom was delayed until the great depths had become favorable to life.

The belts around elevated areas which are far enough from shore to be free from sediment and to have above them a sufficient depth of water to permit the pelagic fauna to reach its full development, are the most favorable spots, and I shall soon show that there is palæontological evidence which indicates that they were seized upon very early in the history of bottom life. It is very probable that colony after colony was established on the bottom, and afterwards swept away, like clouds before the wind, by geological changes, and that the bottom fauna which we know was not the first.

Colonies which started in shallow water were exposed to accidents from which those in great depths were free, and in view of our present knowledge of the permanency of the sea-floor and of the broad outlines of the continents, it is not impossible that the first fauna which settled in the deep zone around the continents may have persisted and given rise to our modern life. However this may be, we must regard this deep zone as the birthplace of the fauna which has survived; as the ancestral home of all the improved metazoa.

The effect of life upon the bottom is more interesting than the place where it began, and we have now to consider its influence in the evolution of animals.

The effect of the secondary acquisition of a sedentary life by modern animals has been fully discussed by many writers, but no one, so far as I am aware, has ever considered the effect of the first settlement of the bottom by pelagic animals, all whose competitors and enemies had previously been pelagic.

It is doubtful whether the animals which first settled on the bottom secured any more food than the floating ones, but they undoubtedly obtained it with less effort, and were able to devote their superfluous energy to growth and to multiplication, and thus to become larger and to increase in numbers faster than pelagic animals.

Their sedentary life must have been favorable to both sexual and asexual multiplication, and the tendency to multiply by budding must have been quickly rendered more active. It is sometimes stated that the capacity for budding has been acquired among the metazoa as the result of a sedentary life, but this view hardly seems to be the true one. Capacity for asexual multiplication is very old, older in all probability than sexual reproduction, and there is no reason to believe that it has ever been lost even by the highest animals, for it must be regarded as nothing more, in ultimate analysis, than discontinuous growth. The tissues of all animals have vegetative power, and external influences determine whether this shall result in continuous or discontinuous growth, and a trace of the power to multiply asexually is retained even among the embryos of mammals. It is therefore wrong to speak of the *acquisition* of a capacity for budding, and it is not at all improbable that the primitive pelagic metazoa multiplied by buds; although the tendency to form connected cormi, and to retain the connection between the parent and the bud until the latter was able to obtain its own food and to care for itself, was a result, and probably one of the first results, of life on the bottom.

The animals which first acquired the habit of resting upon the bottom therefore soon began to multiply faster, both sexually and asexually, than their swimming allies; and their asexual progeny remaining for a longer time attached to and nourished by the parent stock, were much more favorably placed for rapid growth. As bottom animals live on a surface, or at least a thin stratum, while swimming animals are distributed through solid space, the rapid multiplication of bottom animals must soon have led to crowding and to competition, and it soon became harder and harder for new forms from the open water to force themselves in among the old ones, and colonization soon came to an end.

The great antiquity of all the types of structure which are represented among the modern metazoa is therefore what we should expect, for after the foundation for the fauna of the bottom was laid it became, and ever afterwards remained, difficult for new forms to establish themselves.

Our knowledge of the sea-bottom is for the most part from three sources: from dredgings and other methods of exploration; from rocks which were originally laid down beyond the immediate influence of the continents, and from the patches of the bottom fauna which have been gradually brought near its surface by the growth of coral reefs; and from all these sources we find testimony to the density of the crowd of animals on favorable spots.

Deep-sea exploration can give only the most scanty and fragmentary basis for a picture of the sea-bottom, but it shows that its animal life may thrive with the dense luxuriance of tropical vegetation, and Sir William Thomson says that he once brought up at one time on a "tangle," which was fastened to a dredge, over 20,000 specimens of a single species of sea-urchin.

While cruising on the U. S. Fish Commission schooner Grampus, I was interested to find that when a ground-line with baited fish-hooks had been sunk to the bottom in nearly a mile of water, several of the hooks dropped into the mouths of large sea anemones, so that they were brought up uninjured, and were carried more than three hundred miles to the laboratory, where they lived for some time in an aquarium.

The number of remains of palæozoic crinoids and brachiopods and trilobites which are crowded into a slab of fine-grained limestone is most astounding, and it testifies most vividly and forcibly to the wealth of life on the old sea-floor.

No description can convey an adequate conception of the boundless luxuriance of a coral island, but nothing else affords such a vivid picture of the capacity of the sea-floor for supporting life.

The marine plants are not abundant on coral islands, and the animals depend either directly or indirectly upon the pelagic food-supply, so that in this respect their life is like that of animals in the deep sea far from land.

The abundant life is not restricted to the growing edge of the reef, and the inner lagoons are often like crowded aquaria. At Nassau, my party of eight persons found so much to study in a little reef in a lagoon, close to our laboratory, that for four months and more we found new things every day, and our explorations seldom carried us beyond this little tract of bottom. Every inch of the surface was carpeted with living animals, while others were darting about among the corals and gorgonias in all directions; but this was not all, for the solid coral was honeycombed everywhere by tubes and burrows; and, when broken to pieces with a hammer, each mass of coral gave us specimens of nearly every great group in the animal kingdom. Fishes, crustacea, annelids, molluscs, echinoderms, hydroids and sponges could be picked out of every fragment, and the abundance of life inside the solid rock was most wonderful.

The absence of pelagic life in the landlocked waters of coral islands is as impressive and noteworthy as the luxuriance of life upon and near the bottom.

On my first visit to the Bahama Islands I was sadly disappointed by the absence of pelagic animals where all the conditions seemed to be peculiarly favorable.

The deep ocean is so near that, as one cruises through the inner sounds past the openings between the islets which form the outer barrier, the deep-blue water of mid-ocean is seen to meet the white sand of the beach, and soundings show that the outer edge is a precipice as high as the side of Chimborazo and much steeper. Nowhere else in the world is the pure water of the deep sea found nearer land or more free from sediment, and on the days when the weather was favorable for towing outside, we found siphonophores and pteropods and pelagic molluscs, crustacea, salpæ, and all sorts of pelagic larvæ in great abundance in the open sea just outside the inlets.

Inside the barrier the water was always calm, and day after day it was as smooth as the surface of an inland lake. When I first entered

one of these beautiful sounds where the calm, transparent water stretches as far as the eye can reach, and new beauties of islets and winding channels open before one, as those which are passed fade away on the horizon, I felt sure that I had at last found a place where the pelagic fauna of mid-ocean could be taken home alive and studied on shore.

The water proved to be not only as pure as air, but also as empty. At high water we sometimes captured a few pelagic animals near the inlets, but we dragged our surface-nets through the sounds day after day only to find them as clean as if they had been hung out in the wind to dry. The water in which we washed them usually remained as pure and empty as if it had been filtered, and we often returned from our towing expeditions in the sounds without even a copepod or a zoea or a pluteus.

The absence of floating larvæ is most remarkable, for the sounds swarm with bottom animals which give birth every day to millions of swimming larvæ. The mangrove swamps and the rocky shores are fairly alive with crabs carrying eggs at all stages of development, and the boat passes over great black patches of sea-urchins crowded together by thousands, and the number of animals which are engaged in laying their eggs or in hatching their young is infinite, yet we rarely captured any larvæ in the tow-net, and most of those which we did find were old and nearly through their larval life.

It is often said that the water of the coral sounds is too full of lime to be inhabited by the animals of the open ocean, but this is a mistake, for the water is perfectly fitted for supporting the most delicate and sensitive animals, and we had no difficulty in keeping alive, in water taken from the sounds, the surface animals which we caught outside. Even trachomedusæ and doliolums, which are extremely sensitive to impurities in the water, could be kept alive in the house very much better than in any other place where I have ever tried to keep them, and instead of being injurious, the pure water of the sounds is peculiarly favorable for use in aquaria for surface animals.

The scarcity of floating organisms can have only one explanation. They are eaten up, and competition for food is so fierce that nearly every organism which is swept in by the tide, and nearly every larva which is born in the sounds, is snatched by the tentacles around some hungry mouth.

Nothing could illustrate the fierceness of the struggle for food among the animals on a crowded sea-bottom more vividly than the

emptiness of the water in coral sounds. The only larvæ which have much chance of establishing themselves for life are those which are so fortunate as to be swept out into the open ocean, where they can complete their larval life under the milder competition of the pelagic fauna, and while it is usually stated that the pelagic habit has been retained by the larvæ of bottom animals for the purpose of distributing the species, it is more probable that it has been retained on account of its comparative safety.

There can be no doubt, in view of these facts, that competition came swiftly after the establishment of the first bottom fauna, and that it soon became very rigorous and led to rapid evolution; and we must also remember that life on the bottom introduced many new opportunities for divergent modification and for the perfecting of animals.

The increase in size, which came with the economy of energy, increased the possibilities of variation, and led to the natural selection of those peculiarities which improved the efficiency of various parts of the body in their functions of relations to each other, and this has certainly been an important factor in the evolution of complicated organisms.

The new mode of life also permitted the acquisition of protective shells, hard supporting skeletons, and other imperishable structures, and it is therefore probable that the history of evolution in later times gives us no index as to the time which was required to evolve, from pelagic ancestors, the oldest animals which were likely to be preserved as fossils.

Life on the bottom also introduced another most important influence in evolution—competition between blood relations. In the animals which we know most intimately, divergent modification, with the extinction of connecting forms, results from the fact that the fiercest competitors of each animal are its closest allies, which, having the same habits, living upon the same food, and avoiding enemies in the same way, are constantly striving to hold exclusive possession of all the essentials to their life. When a stock gives rise to two divergent branches, each of them escapes competition with the other, so far as they differ in structure and habits, while the parent stock, competing with both at a disadvantage, is exterminated.

Among the animals which we know best, evolution leads to a branching tree-like phylogeny with the topmost twigs represented by living animals, while the rest of the tree is buried in the dead past. The connecting form between two species must, therefore, be constructed in imagination or sought in the records of the past.

Even at the present day things are somewhat different in the open ocean, and they must have been very different in the primitive ocean, for a pelagic animal has no fixed home, one locality is like another, and the competitors and enemies of each individual are determined, in great part, by accidents. We accordingly find, even now, that the evolution of pelagic animals is often linear instead of divergent, and the early steps in the series often live on side by side with the later and more evolved forms. The radiolarians and the medusæ and the siphonophores furnish many well-known illustrations of this feature of pelagic life.

No one is much surprised to find in the South Pacific or in the Indian Ocean a salpa, or a pelagic crustacean, or a surface fish which has previously been known only in the North Atlantic, and the list of species of marine animals which are found in all seas is a very long one. The fact that pelagic animals are so independent of those laws of geographical distribution which limit land animals is additional evidence of the easy character of the conditions of pelagic life. We have seen that one of the first results of life upon the bottom was to increase asexual multiplication and to lengthen the time during which buds remained united to and nourished by their parents. One result of this is the crowding together of individuals of the same species, and competition between relations. We have in this and in other obvious peculiarities of life on the bottom a sufficient explanation of the fact that, since the first establishment of the bottom fauna, evolution has resulted in the elaboration and divergent specialization of the types of structure which were already established, rather than in the production of new types.

Another result of the struggle for existence on the bottom was the escape of varieties from competition with their allies by flight from the crowded spots and a return to the open water above; just as in later times the cetacea and sea-birds have gone back from the land to the ocean. These emigrants, like the civilized men who invade the homes of peaceful islanders, brought with them the improvements which had come from fierce competition, and they carried everything before them and produced a great and rapid change in the character of the pelagic fauna.

The rapid intellectual improvement which has taken place among the mammalia since the middle tertiaries, and the rapid structural development which took place in animals and plants when the land fauna and flora were first established, are well known, but the fact that the discovery of the bottom initiated a much earlier, and probably much

more important era of rapid development in the forms of animal life has never received the attention which it so well merits.

If the views which I have advanced are correct, the primitive bottom fauna must have had the following characteristics:

1. It was entirely animal, without plants, and it at first depended directly upon the pelagic food-supply.

2. It was established around elevated areas in water deep enough to be beyond the influence of the shore.

3. The great groups of metazoa were rapidly established from pelagic ancestors.

4. There was a rapid increase in the size of the bottom animals and hard parts were quickly acquired.

5. The bottom fauna soon produced progressive development among pelagic animals.

6. After the establishment of the bottom fauna, elaboration and differentiation among the representatives of each primitive type soon set in and led to the extinction of the connecting forms.

There is no reason to suppose that the first animals which were adapted for preservation as fossils have been discovered, and many of the oldest fossils, like the pteropods, are most certainly the modified descendants of simpler ancestors with hard parts, but it is interesting to note that the oldest fossil fauna which is known to us is an unmistakable approximation to the primitive bottom fauna as I have outlined it.

Walcott has given the following sketch of the broad general characteristics of the lower cambrian fauna:

The lower cambrian fossils are distributed through strata which, in Washington and Rensselaer counties in New York, are nearly two miles thick, and some of them, at least, were deposited in water of considerable depth. This is shown by the fineness of the sediment and by the perfect preservation of tracks and burrows in soft mud and of soft animals like jelly-fishes. These show that the sediment was laid down slowly and gently, in water so deep as to be free from disturbance, and under conditions so favorable that it contains the remains of some animals which are not found again until we reach a very much more modern period. The fossil medusæ of the lower cambrian are so perfect that their identity is unquestionable, yet it is not until the Solenhofen lithographic slate of the Jura is reached in ascending the geological scale, that medusæ are again met with; and corals and lamellibranchs are found in the lower cambrian, although as they are not found again until the

silurian rocks are reached, we have no record of their existence through the long period covered by the middle and upper cambrian.

The fauna of the lower cambrian, while it undoubtedly lived in water of very considerable depth, was not oceanic but continental, and Walcott says that "one of the most important conclusions is, that the fauna of the lower cambrian lived on the eastern and western shores of a continent that in its general configuration outlines the American continent of to-day. Strictly speaking, the fauna did not live upon the outer shore, facing the ocean, but on the shores of interior seas, straits, or lagoons that occupied the intervals between the several ridges that ran from the central platform east and west of the main continental land-surface of the time."

The lower cambrian fauna was rich and varied, but it was not self-supporting, for no fossil plants are found, and the primary food-supply was pelagic. Animals adapted for a rapacious life at the surface, such as the pteropods, were abundant, and they prove the existence of a rich supply of pelagic animals. All the forms are either carnivorous animals, such as medusæ, corals, crustacea and trilobites, or they are adapted, like the sponges, brachiopods and lamellibranchs, for straining minute organisms out of the water, or for gathering up those which rained down from above, and the conditions under which they lived were obviously very similar to those on the bottom at the present day.

Walcott's studies show that the earliest known fauna had the following characteristics:

1. So far as the record goes it consisted of animals alone, and these animals were dependent upon the pelagic food-supply for support.

2. While small in comparison with many modern animals, they were gigantic in size as compared with primitive pelagic animals.

3. The species were few, but they represented a very wide range of types.

4. All the types have modern representatives, and most of the modern types are represented in the lower cambrian.

5. The habitat was not the bottom of the deep ocean, but the submerged surface of a sinking continent, under water of considerable depth.

Remains of bottom animals are found in rocks below the cambrian, and Walcott believes that while the olenellan fauna adds a little more to our knowledge of the rate of convergence backwards in geological time of the lines representing the evolution of animal life, it also proves, at

the same time, that an immense interval has elapsed between the beginning of life and the epoch represented by the olenellan fauna. He says: "That the life in the pre-olenellus seas was large and varied, there can be little, if any, doubt. The few traces known of it prove little of its character, but they prove that life existed in a period far preceding lower cambrian times, and they foster the hope that it is only a question of search and favorable conditions to discover it."

No one can question the validity of the basis for Walcott's hope, for pelagic animals have undoubtedly established themselves on the shores of elevated tracts again and again, during the oscillations of the sea-bottom, and we have every reason to expect and look for their remains.

If, however, it is true that the primitive stem-forms were pelagic and minute, there is little hope of finding their delicate microscopic remains in the sedimentary rocks of the shore.

The cambrian fauna is usually regarded as a half-way station in a series of organisms which reaches back into the past for an immeasurable period, and it is even stated that the history of life before the cambrian is longer, by many fold, than its history since.

So far as this opinion rests on the diversity of types in cambrian and silurian times it has no good basis, for if the view which I have advocated is correct, the evolution of the ancestral stem-forms took place at the surface, and all the necessary conditions for the rapid production of types were present when the bottom fauna first became established.

As we pass backwards towards the lower cambrian we find closer and closer agreement with the biological conception of the primitive life at the bottom.

We cannot regard the olenellan fauna as the first bottom fauna, for it contains forms which have been secondarily adapted for a pelagic life, such as the pteropods.

We may, however, feel confident that the first bottom fauna resembled that of the lower cambrian in its physical conditions, and in its most distinctive peculiarity, the abundance of types and the slight amount of differentiation among the representatives of these types.

Far from seeing in the lower cambrian fauna a half-way station in a long series of bottom animals, the biologist must regard it as an unmistakable and decided approximation to the primitive fauna of the bottom, beyond which life was represented only by simple and minute pelagic organisms.

CHAPTER VIII.

THE ORIGIN OF THE CHORDATA, CONSIDERED IN ITS RELATION TO PELAGIC INFLUENCES.

SECTION 1.—*The Ancestral Chordata.*

I shall now attempt to study the origin and significance of the structure of appendicularia in accordance with those conditions which must, as Dohrn has pointed out (Studien, etc., VIII, p. 79), direct all inquiry into the genealogy of animals.

All biologists will agree with Dohrn that no amount of morphological information, or of exhaustive microscopical study of the structure and development of animals, can suffice, in the absence of comprehensive knowledge of their mode of life and of the conditions of their existence, for the institution of inquiries into their phylogenetic relationship.

Unquestionably the first condition for genealogical inquiry is, as Dohrn says, the establishment of a direct connection between our morphological studies and the facts of physiology and biology.

"The homologies which are established by comparative anatomy, and the primititive identities which are established by comparative embryology, are only the means for this end. They are in themselves valuable in phylogenetic inquiry only so far as they furnish us the opportunity to pass from the consideration of the structure of organs as they now exist, and of the functions of these organs at the present time, to the consideration of conditions which have passed away ; to the study of the history of the modifications which have come between these structures and functions and those which we must attribute to the same organs at an earlier genealogical stage."

Keeping these conditions of genealogical inquiry in view, let us try to study the structure of appendicularia in relation to the conditions of its life, so far as these are known to us, and let us see what functions we must, according to the principle of change of function, attribute to the organs of the remote ancestors of the tunicates, and what are the paths these organs have traversed in reaching their modern structure.

If the reader of the following pages should think that I wander too

far from the beaten paths of observation, I must plead as my excuse that the study of phylogeny is impossible without the use of the imagination, and that the field is already occupied by a phylogeny of the tunicata which cannot be set aside until a more satisfactory one has been found.

Appendicularia is a very simple organism, and while much ingenuity has been expended in the negative task of accounting for the absence of all the structures which it lacks, I hope that the more positive attempt to account for its actual structure will not lead us into any great difficulties.

In the belief that the sequel will justify the assumption, I shall, as my starting-point, picture the ancestor of appendicularia as a simple, minute, unsegmented, chordate animal, leading a free, locomotor, pelagic life and subsisting upon the micro-organisms of the ocean. I shall also assume that this ancestor had an elongated, unsegmented body stiffened by an axial, unpaired, unsegmented notochord, like that of amphioxus, appendicularia, and the ascidian larva; that it had a simple, elongated, dorsal, nervous system, and an elongated, ventral, digestive tube, without pharyngeal clefts; that this tube was nearly straight; that it had a capacious lumen, and that, as in amphioxus and the tunicates, this was permanently distended and ciliated, and that the water, with the micro-organisms that float in it, was swept through it by endodermal cilia and not by muscular contractions.

In order to entangle the floating particles of food and to hold them while the water swept on through the intestine and out of the anus, gland-cells for the excretion of slime were scattered among the ordinary ciliated endoderm cells of the digestive tract. In origin, these slime-cells may have been modified or specialized digestive gland-cells.

As particles which are entangled and held captive near the oral end of the gut are more perfectly exposed to its digestive action than those which continue to float with the stream, the most anterior slime-cells are most efficient and valuable, and as each variation in this direction gave its possessor an advantage, the slime-cells gradually, through the action of natural selection, became localized in the pharyngeal region, and this region gradually became enlarged and was thus set apart, at a very early period, as a specialized tract of the gut.

It is also probable that, at a very early stage in the phylogeny of these primitive chordata, a blind pouch was developed, behind the pharynx, to catch the food-particles as they were hurried past with the stream of water and to retain them long enough for perfect digestion, and that the rudiment of the organ which has in the higher vertebrates become the liver was thus established.

In these primitive animals the current of water through the digestive organs was most useful as the vehicle for floating food, but while necessary, it was a necessary evil, for the large distended lumen which furnished it a channel also permitted undigested food to be swept away and lost.

The immovable, permanently distended, ciliated digestive tract of a modern lamellibranch is very similar to that of these primitive chordata, but the lamellibranchs have acquired an apparatus for straining off the water from the captured food, so that the digestive tract is relieved from this disadvantageous current.

If, after the pharynx had been established, a secondary opening from it to the exterior were to be formed, this opening would permit the water to escape without passing through the intestine, and as the advantage of this new arrangement is obvious, there can be no doubt that after an opening of this sort was once formed, it would be preserved and perfected by natural selection, as a channel for the escape of the water after the food has been strained out and entangled by the excretion of the pharyngeal slime-glands.

I shall show, further on, that if an useful opening of this sort were to be fixed and preserved by natural selection on one side of the body, the laws of growth would soon cause it to be duplicated on the other side. These two openings are the so-called gill-slits of appendicularia, although they are beyond question much older than the modern appendicularia, dating back to a time before this animal had acquired the features which distinguish it from its more primitive chordata ancestors.

I am not able to suggest what led to the first establishment a of secondary opening into the pharynx; but, once formed, its preservation and gradual improvement, by natural selection, as a channel for the escape of superfluous water, and its duplication on opposite sides of the body, are easily intelligible.

If we accept the view that the chordata type was evolved under purely pelagic influences, we are forced to believe that the first chordata were minute, and that their small bodies were soft, and unprotected by a hard covering. If we also admit that their digestive tract was a channel for a current of water, we can hardly believe that they needed respiratory organs, or, for that matter, excretory organs, for all the tissues of a minute soft animal, bathed within and without by pure water, must have been sufficiently aerated and purified without any organs for this purpose.

It is not at all probable, then, that the pharyngeal clefts were originally either gills or renal organs, and we have seen that the conditions of pelagic life furnish a much more simple explanation of their advantage, and I believe that the view that they were originally concerned in nutrition rather than in respiration will commend itself to all who approach the subject without any philosophical preconception.

After they were once established they gradually effected a rearrangement of the slime-cells and ciliated cells of the pharynx, for as it now became important that all the food particles should be entangled by the product of the slime-cells before it reached the pharyngeal clefts, the slime-cells were gradually restricted to the anterior part of the pharynx, while the ciliated cells gradually became specialized to carry the entangled food past the openings and to convey it safely into the œsophagus.

All the parts of the pharynx of appendicularia are beautifully constructed for this purpose. The pharyngeal clefts are situated far back in the pharynx, and are separated by nearly its whole width from the œsophagus. They are fringed by large cilia to expel the water, and they are separated from each other by a vertical shelf or velum on the ventral floor of the pharynx, so placed as to prevent cross-currents.

In front of this shelf the slime-cells are brought together in two rows, near the middle line, just inside the mouth, to form the hypopharyngeal band or endostyle. Between these two rows of slime-cells there is a median row of large ciliated cells, so placed that they drive the slime forwards to the point where a ciliated peripharyngeal band receives it and carries it up each side of the pharynx just behind the mouth, into the most favorable place for entangling the food, as this enters with the current of fresh water.

On the dorsal middle line the threads of slime are gathered up and guided along the epipharyngeal band or dorsal lamella, beyond the influence of the current of water which sets backwards, on each side of the ventral velum, to the pharyngeal clefts, and the food is thus safely conducted into the œsophagus while the water escapes.

Up to this point I believe that the ancestral history of the tunicates was identical with that of the vertebrates, for the hepatic cæcum, the dilated pharynx, the pharyngeal clefts, the hypopharyngeal gland and the peripharyngeal bands have been inherited by all the chordata, and have impressed themselves so firmly in their organization that even the highest vertebrates still retain them, either as vestiges, or as organs which have been fitted to new functions.

I believe, however, that while they were acquired before the tunicates diverged from the chordata stem, they were acquired by an organism whose environment and habits of life were essentially like those of the modern appendicularia.

All the parts of the pharynx of appendicularia are so beautifully coordinated for effecting a purpose so useful and so well adapted to the conditions of its simple pelagic life, that we find it difficult to resist the belief that its ancestors had essentially the same habits, and that they lived under essentially the same conditions, and that this simple organization was directly acquired in adaptation to these conditions.

If this view involved any great or unusual difficulties we might well distrust it, notwithstanding its simplicity; but I shall try to show that it does not. In the preceding chapter I have shown that it accords with our knowledge of the fundamental principles of the general biology of the ocean, and further on I shall try to show that it is equally in accord with the principles of morphology.

At present we must devote our attention to the history of the evolution of the tunicates from this primitive chordata stem.

SECTION 2.—*The Origin of the Tunicates.*

Like most recent students of the tunicates, I believe that we have in appendicularia a persistent representative of the primitive tunicata; but, unlike many of them, I fail to find in its structure any evidence of degeneracy, or in its habits any basis for the assumption that it is degenerated. In most respects its structure is like that of the hypothetical ancestor whose evolution we have traced. It has an unsegmented notochord, and a capacious lumen throughout the whole course of the digestive tract from mouth to anus. This lumen is permanently distended and food is carried through it by cilia. It has a blind diverticulum from the stomach, and the greatly expanded pharynx opens laterally through two ciliated pharyngeal clefts, through which the water escapes while the food passes into the œsophagus. There is a ventral slime-gland just inside the mouth, and its excretion is conveyed upwards around the pharynx by the cilia of the peripharyngeal bands, and is then swept into the œsophagus with the entangled food.

This increasing complexity and perfection of the pharynx is accompanied by an increase in its size, so that in the primitive tunicates it soon comes to be the most important and dominant organ of the body, and

brings about adaptive changes in other parts.   One of these is the differentiation of a stomach for the retention and digestion of the food, in the direct course of the gut.   As long as the food was mixed with great quantities of water, digestion and assimilation probably went on simultaneously in all parts of the post-pharyngeal gut, but as the water found another exit and the food thus became more compact and solid, the stomach of appendicularia became established and thus divided the gut into an œsophageal, a gastric, and an intestinal region.

Our knowledge of the primitive vertebrates seems to me to be too scanty to show whether this differentiation occurred before or after the tunicates diverged from the ancestors of the vertebrates.   We are now concerned with the history of the tunicata line alone, and the fact that the differentiation now exists in all tunicates shows that it was brought about very early in their history.

Another most important change in the relations of the gut also took place very early in their history.   The intestinal portion became bent upon the enlarged pharynx so as to form a ⊐ with the intestinal bar of the ⊐ ventral to the pharyngeal portion, and with the anus on the ventral middle line under the pharynx.   Herdman represents the primitive condition of the digestive tract of tunicates as a ⊐, with the intestine and anus dorsal instead of ventral (page 128); but I shall show further on that the relations exhibited by appendicularia are the primitive ones, from which we must derive those which are exhibited by other tunicates.

By this change the tail was freed from the gut and was made much more efficient as an organ of locomotion, while the fæces were discharged from the anus into the current of water which set out through the pharyngeal clefts.   This latter feature may not have been of any value so long as habits of active locomotion were retained, but, as we shall see, it became very important at a later stage.

The embryology of the ascidians shows that this arrangement of the digestive tract was secondary; that at one time it was straight, extending into that region of the body which is now specialized in appendicularia as a tail.   The advantage to an active pelagic animal of this change is obvious, since it permits the tail to become purely locomotor.   As each slight variation in this direction must have given a slight increase in the freedom of movement, the shape of the body of appendicularia is easily intelligible as the result of natural selection, and while the change is complete in this, the most primitive tunicate which we know, so that we can only conjecture the transitional stages, the

change itself is not a complicated one. It presents little difficulty, although the resulting differentiation of appendicularia into two regions or "segments," a body and a tail, has been made the basis of much speculation.

The great development of the pharynx and the reduction of the tail to an organ of locomotion soon resulted in a pronounced change, of the sort for which Dana long ago proposed the term *cephalization*.

As the functions of the pharynx, and of its oral end in particular, became more and more complicated and more and more exactly co-ordinated, while those of the tail became simplified, the elongated nervous system became differentiated in a corresponding way, and its caudal portion became reduced to a caudal nerve, while its oral extremity became evolved into a cerebral vesicle with sense-organs and nerves in relation with the co-ordinated structures of the pharynx.

All the characteristics of appendicularia, except the structure of the heart and the structure and position of the reproductive organ, are thus seen to be intelligible as direct adaptations to a pelagic life; for its distinctive features, as compared with other primitive chordata, are the U-shaped folds of the digestive cavity, the sharp separation of the tail from the body, and the differentiation of the nervous system into a caudal nerve and anterior vesicle.

We have little basis for speculation as to the path by which the reproductive organ acquired its present position, and it is by no means certain whether the tunicate heart is homologous with that of the other chordata.

The conditions of pelagic life are so permanent that we may safely make use of the structure and habits of the modern pelagic forms to reconstruct this part of the ancestral history of the tunicates, for time writes no wrinkles on the azure brow of the ocean.

As regards the later history the case is different. Between appendicularia and the ascidians there is a great gap which we can bridge only in imagination. The transitional animals are totally unknown, and the conditions of life on the bottom of the modern ocean may, possibly, be very different from those which prevailed when the fixed ascidians were first evolved.

It is easy to imagine changes which might have gradually converted an ancestor like appendicularia into a descendant like the fixed ascidians, through successive adaptations to a sedentary life, but in the absence of all evidence we cannot feel implicit confidence that the imaginary picture bears any minute and detailed resemblance to the actual history.

It seems probable that after the bottom of the ocean became fit for life, some of the descendants of the primitive pelagic tunicates gradually acquired the habit of sometimes swimming upon or near it in an inclined position with the mouth downwards to suck up the organic sediment, and that they also acquired the habit of resting upon the bottom in this position.

We may well doubt whether these animals obtained any more food than their pelagic ancestors, but it is well known that it is not the amount of food, but the ratio between the supply and the amount of expended energy which affects size. As this new habit economized energy both during rest and during activity, it permitted an increase in size, and it is interesting in this connection to note that Chun has found at great depths appendicularias which may well be called gigantic as compared with all which are known to exist at the surface.

With each increase in size, the habit of visiting the bottom must itself have become more and more fixed, until the life upon the bottom, which may have been at first only intermittent and more or less accidental, at last became established in the ancestors of the ascidians as a constant characteristic peculiarity.

As this new mode of life was gradually acquired, some method of aerating the fluids of the body must also have been gradually evolved; for without it, a minute animal adapted for a free active life in the highly aerated surface-water, could not, at the same time that it grew larger, acquire a less active habit of life in the bottom strata where the water is less perfectly aerated, the products of decomposition of organisms more concentrated, and the capacity for passing from exhausted and impure water to a fresh environment, restricted both by the more stationary habit and by the fact that life in space has been exchanged for a home which is limited by a surface.

Undoubtedly the change of habit was accompanied by the gradual perfecting of the system of blood-spaces around the pharynx, which, at first indefinite and irregular, became constant on the margins of the pharyngeal clefts, which thus gradually acquired a new function and became gill-slits, and also became duplicated as the animals grew larger and the need for more perfect respiration increased with their change of habits.

I hope that no one will interpret the last sentence as an expression of the belief that the need for respiration caused the gill-slits to multiply. I believe, and shall try to show further on, that the tendency to dupli-

cate a structure, either radially, bilaterally or serially, is a result of the method of growth by cell multiplication, and that in the case in question the serial reduplication has been fixed and preserved by natural selection on account of its value in respiration.

The context shows that I also regard the gill-slits of vertebrates and those of tunicates as homologous structures inherited from a common source, the primary pharyngeal clefts; but that I regard the increase in their number as a secondary change which has occurred in both lines after their genealogical paths had diverged.

It does not seem necessary to defend the thesis that the number of gill-slits in the ascidians is the result of secondary multiplication, since, as I shall show further on, it is accepted by Dohrn (Studien, etc., IX, 417), who has proved himself a most rigorous critic of the logic of morphology.

There is reason to believe that the multiplication of gill-slits in the tunicates has not only taken place independently, but that it has taken place in a peculiar way. Anatomy and embryology give evidence that while the perforations of the tunicate pharynx multiplied, the perforations of the outer wall of the body did not; and that the external portions of the two primary clefts became distended into a pair of spacious perithoracic chambers, each with numerous ciliated openings into the pharynx, and a single opening to the exterior which perhaps became enlarged as the gill-slits multiplied.

So long as the primary function of the first pair of pharyngeal clefts, the discharge of the superfluous water, was the only one, they probably remained circular like those of appendicularia; but as they became concerned in respiration and increased in number, and were furnished with definite blood-vessels, they became elongated vertically and, forming a series side by side over a considerable area on each side of the pharynx, they thus became much more efficient organs for the aeration of the blood.

In this simple way metamerism, that fetish of the morphologists, was established among the tunicates, and there is no evidence that it has ever involved any of their organs except the gill-slits and the pharyngeal blood-vessels.

A vertical series of slits, elongated longitudinally, would undoubtedly have permitted the water to escape just as well as a longitudinal series elongated vertically, but it is possible that, during the gradual establishment of the respiratory circulation, those of the irregular and variable

blood-spaces which were most nearly transverse to the current of water from the mouth to the primary clefts, were the ones which were first made definite by natural selection, and that the arrangement of the gill-slits was thus determined.

We can only conjecture how this unknown ancestral swimming organism first became fixed, but the discovery of its descendants on the modern sea-floor is among the possibilities of future explanation.

The sedentary habit undoubtedly came gradually, and at first it may have been temporary, confined perhaps to the breeding season, when, loaded down with eggs, the animal may have learned to rest upon the fragments of crinoids, or the shells of trilobites or brachiopods or molluscs, to avoid clogging its delicate ciliated and vascular pharynx with sediment. At the point where the heavy anterior end of the tadpole-shaped body rested, the ectoderm cells, just below the mouth, probably became modified for the excretion of an adhesive cement.

The sedentary habit, which must have resulted in a still greater economy of energy and a corresponding increase of size, undoubtedly became more and more firmly established, and the changes which followed and resulted in the evolution of the ascidian type are easily intelligible as adaptations to a fixed home, although we have little to show the sequence of their acquisition.

So long as the animal led a free life the fate of the deoxidized water after it left the gill-slits had no meaning, but with the fixed habit came the need for driving it away as far as possible, and the external apertures of the perithoracic chambers became small, moved towards each other, and finally united to give to the exhaled current the strength of concentration. The attitude of the animal upon the bottom undoubtedly determined the dorsal instead of ventral location of the common aperture and of the median atrium or cloaca. As each step in this process of concentration must have been advantageous, its evolution by natural selection is easily intelligible. The accumulation of fæces from the intestine, around a fixed animal, is so unsanitary that the anus has disappeared in many sedentary metazoa, while in others, such as the crinoids and the lamellibranchs for example, secondary adaptations for sweeping away the refuse matter have been acquired.

The folding of the originally straight digestive tract of the primitive chordata into a U with the anus and intestine ventral to the pharynx, took place in the ancestral tunicates as an adaptation to locomotion, but, as appendicularia shows, it incidentally brought the anus into the

region of the pharyngeal clefts.   As the sedentary habit became slowly established the anus became shifted from the middle line into the exhaled current from the left perithoracic chamber, and finally into the margin of its aperture, so that, during the migration of the exhalent openings, the U of the digestive tract became twisted into an 8 in such a way that, as Plate VIII, Fig. 2 shows, the intestine p passed on the left side of the œsophagus, q, to open dorsally into the atrium, near the middle line, but a little to the left.

This arrangement of the digestive organs is very characteristic of the tunicates, and the few exceptions are clearly due to later changes. Thus in doliolum the atrium has moved backwards as an adaptation to locomotion, and the anus has followed it until the gut has become nearly straight.   The intestine and anus of the adult aggregated Salpa pinnata, Plate I, Fig. 1, are ventral; but I have shown that in the young the intestine crosses to the left of the œsophagus to open dorsally, as it does in the adults of all ordinary salpæ.   In the Polyclinidæ the loop of the intestine has been elongated, with the elongation of the body, until the bend of the 8 has been obliterated, and the presence of the characteristic 8 in more primitive ascidians such as clavelina shows that the Polyclinidæ have been more recently modified.

All sedentary animals which take their food by means of cilia have their apertures raised in some way above the reach of sediment.   In the crinoids this end is reached by a stalk; in the lamellibranchs it is attained either by siphons, or by the vertical elongation of the shell as in the oyster; and the shifting of the area of attachment of the ascidians from the oral end to the aboral end, the elongation and approximation of the mouth and the atrial aperture, the acquisition of oral and atrial sphincter muscles, the degeneration and disappearance of the locomotor tail, and the simplification of the nervous system, are such obvious adaptations to a sedentary life that it is not necessary to discuss them.

SECTION 3.—*The Annelidian Hypothesis.*

I believe that the structure of the tunicates has been acquired as an adaptation to the biological conditions which prevailed at the surface of the primitive ocean, and that it has been evolved by the gradual addition of successive complications on to the body of a still more primitive and simple ancestor.   This involves the total rejection of the dogma that the vertebrates are modified annelids, and that the tunicates are degenerated vertebrates.

While it is not my purpose to discuss the ancestral history of the vertebrates, the remote phylogeny of the tunicates is unquestionably identical with that of the other chordata, and I cannot ignore the general acceptance of an opinion which is absolutely irreconcilable with the one which I have presented.

This prevailing opinion has interwoven itself with the literature in such a complicated way that one may well shrink from the interminable labor which the critical revision of the whole of it would involve. I myself decline to undertake what I regard as an unprofitable and useless task; unprofitable, as the literature rests on an untenable and false basis, and useless, since I do not hope to induce those who have stored their minds with the endless details of morphology docketed and pigeon-holed according to a false system, to unload all this rubbish and to build again on a new foundation.

I shall therefore restrict myself to a discussion of the origin of the two most characteristic systems of tunicata organs, the gill-slits, and the pharyngeal ciliated cells and gland cells; and I shall here confine myself to the observations and reflections of a single writer, Dr. Dohrn.

I make this selection the more willingly, as Dohrn's name is most intimately associated with the annelidian hypothesis, and because his writings are not only the ones which have been most influential, but also the ones which are most comprehensive and most attractive to the reader.

The "Ursprung der Wirbelthiere" is a most fascinating book. Soon after it appeared I placed it in the list of works which my students are advised to read, and for many years an acquaintance with it has been expected of all who have been examined for the degree of Ph. D. in the Johns Hopkins University.

My students have even prepared for their own use an English translation of it, and I have read it with them several times with interest and pleasure. At the first reading my pleasure was almost that of conviction, but as the ingenious details became familiar, and the essay was more sharply focused in its completeness, and was held, as it were, at arm's length, so that the whole picture could be seen at one view, I have read it, as I have read Gulliver's Travels, with admiration for the skill which has elaborated it in such logical minuteness from a fundamental assumption which is purely imaginary.

The story, as told by Dohrn in the "Ursprung," is so consistent and logical that I see no reason why animals like the tunicates might not have been evolved in the way which he pictures so vividly, although I

believe that the actual tunicates have been produced in a very different way.

I shall therefore examine the account of the origin of the gill-slits which Dohrn gives in the "Ursprung," and the view of the ciliated and glandular structures of the pharynx which is developed in his "Studien," especially in Parts VII, VIII and IX, in order to determine how far the origin of these structures is accounted for by the annelidian hypothesis, and what superiority, if any, this has over the much simpler hypothesis which is here advanced.

Dohrn says (Ursprung, p. 10) that the branchial apparatus of the tunicates and that of balanoglossus *are so much more complicated* than that of the selachians, and their origin is so much more difficult to understand, that they are of no help to us in our attempt to trace the origin of gill-slits.

I am quite at a loss for the meaning of this passage, for no secondary perforation of the pharynx could possibly be less complicated than the gill-slits of appendicularia, nor could it be developed in a simpler way than by the involution of a pit on the side of the body.

It is quite true that we do not know how the gill-slits of appendicularia first came into existence, or what influence led to their formation, but their usefulness as channels for the escape of the water which, before they were formed, must have passed through the intestine, is clear, and we can understand why they have been preserved, by natural selection, on account of this advantage.

We are forced to believe that the pharynx did, in some way, acquire a secondary communication with the exterior, although we are not able to say how it was acquired.

Dohrn's view of the origin of gill-slits is based upon the need for an explanation of the original formation of the perforation. He says (Ursprung, p. 10): "What is a gill-slit? Perforations of the body-wall do not take place directly, and still less do they form connections with corresponding perforations of the digestive tract," and he therefore undertakes to study the origin and primitive function of gill-slits by the aid of the law of the change of function, and to find in a more primitive function an explanation of their present function as channels for water.

As his point of departure is the need for an explanation of the origin of the perforation, we feel a natural hope that we are to be led to this explanation, but this hope ends in disappointment.

He regards the gill-slits as modified segmental organs, but he tells us

explicitly, on page 10, that "we are not able to assign any reason why segmental organs should unite with the gut," and his explanation of the origin of the perforations is no explanation at all, since it simply assumes, but does not account for, the very phenomenon which it is supposed to make clear.

His inability to understand the direct origin of the secondary perforations of the gut has one most remarkable result, for the view that the gill-slits are segmental organs involves the view (Ursprung, p. 57) that the anus of the tunicates is not a primary anus nor a secondary one, but a tertiary one, and that the ancestors of the tunicates have not only acquired two new secondary anal apertures, but that they have lost one mouth and acquired a second, and that they have lost this and acquired a third. As these mouths are supposed to be modified segmental organs, we are, according to the acknowledgment on page 10, "unable to assign any reason why they should have united with the gut."

The original mouth of the ancestors of the chordata was, according to Dohrn (page 3), on what is now the dorsal surface, and the primitive œsophagus passed through what is now known as the fossa rhomboidea of the brain.

This ancestral mouth degenerated and disappeared as it was gradually superseded in the remote progenitors of the vertebrates by a second mouth (page 5), which is the mouth of the vertebrates of the present day, and of the ancestors of the tunicates (page 57) as well, although it was gradually converted first into a sucker, and finally into an organ for fastening the tunicata to foreign bodies, while these animals gradually acquired a tertiary mouth (page 58) by the formation of a secondary communication between the nasal chamber and the gut.

Dohrn says (page 60) that these assumptions "set the relation between the fishes and the ascidians in the right light," although the perforation of the gut, which the hypothesis is to explain, is not only left unaccounted for, but is multiplied so many times that, like the man with an unclean spirit, its last state is worse than the first.

Dohrn says that the secondary nature of the mouth of the vertebrates is proved by its very late appearance in the young vertebrate after its embryonic body and its great systems of organs are fully formed, and by the fact that, when it does make its appearance, it does not lie at the anterior end of the body, in the place which it finally occupies in the great majority of vertebrates, but at a spot some distance behind this place.

It is not possible to attach much weight to either of these arguments, for slight changes in the position of organs are not unusual, and it is well known that the ontogenetic acceleration or retardation in the relative time of appearance of structures is by no means exceptional, and it would be as safe to assume that the change in the pitch of the voice of man is phylogenetically older than the sexual maturity of the ancestors of man, as it is to assume, from the same sort of evidence, that the aortic system of vertebrates is older than the mouth.

The vertebrate mouth unquestionably bears a great morphological resemblance to a pair of gill-slits. As Dohrn points out, it is bordered, like the gill-slits, by a pair of visceral arches, it lies in front of the first pair of true gill-slits, it arises at the same time with them in the embryo, and like them it opens into a section of the gut.

A ventral view of a shark shows the resemblance between the mouth and the true gill-slits in the most impressive way, and if any pair of them were to be united with each other at their ventral ends, they would become perfectly equivalent to the mouth. The armature of the mouth is repeated on the gills, and there is reason to believe that the jaw-arches have at one time carried gills like the gill-arches.

This resemblance is not imaginary. Beyond all question it is real, and it is certainly most remarkable and suggestive, but does it prove that the vertebrate mouth is phylogenetically a pair of gill-slits?

When, in my student days, my instructor held before me the skull of a turtle and called upon me to observe the centrum, the transverse processes and the neural arch of the occipital vertebra, I was, for the time, convinced that the occipital bone had arisen by the differentiation and specialization of a bony vertebra, like those in the neck of a turtle, and that its history had been identical with that of the thoracic vertebræ, which have been differentiated and specialized in the same way into constituent parts of the bony box which covers the body of the turtle, as the skull covers the brain.

In all these cases the morphological resemblance is undeniable, but our opinion of its phylogenetic significance depends upon our view of the nature and origin of the metamerism of vertebrates, a question which will soon be discussed.

At present we must confine ourselves to a narrower point of view, and learn where we are led by Dohrn's opinion that the vertebrate mouth is actually a pair of gill-slits.

If the present mouth of the vertebrates was once a pair of gill-slits,

the ancestors of the vertebrates must have had at that time another mouth, and during the long series of stages of development, while the gill-slits were gradually assuming the function of a mouth, food must have been taken in through both openings; for the new function of the gill-slits must have been acquired slowly alongside their old function, until the new mouth finally became so perfectly adapted for its new function that it supplanted and replaced the old one.

According to Dohrn, these considerations force us to believe that the primitive mouth of the ancestors of the vertebrates and of the tunicates was situated in the fossa rhomboidea, where an œsophagus pushed inwards to join the mid-gut, in the same way that it is joined in insect embryos by the fore-gut. This primitive mouth and its œsophagus were homologous with the corresponding organs of modern arthropods and annelids. The mouth of the modern vertebrates is then to be regarded as a secondary mouth, which has gradually supplanted and replaced the old one on account of its greater efficiency.

It follows from this, according to Dohrn (p. 56), that the "so-called larva" of the ascidians is a degenerated fish, and that all the features which show the derivation of the cyclostomes from the fishes show also that the process of degeneration has reached its extreme in the tunicates. The cyclostomes are held to owe their degeneracy to parasitism, and the most important element in the more advanced process of degeneration is that the ascidians no longer fasten themselves to fishes nor make use of their bodies as food, but that they fasten themselves to stones, to ships, or to the bodies of other animals which do not serve as food, such as the shells of crabs or the tubes of annelids.

The mouth (p. 57) which in the cyclostomes serves both as an organ for attachment to the skin of fishes, and also as a sucker for extracting their blood, has become converted in the ascidians into an organ for attachment; and these animals have thus lost their old mouth, which was homologous with that of the true vertebrates, and have acquired a new one which is homologous with the vertebrate nasal chamber.

The process, Dohrn says, must be represented as follows: The fishes take in the water for respiration through the mouth, but as this is used by the parasitic cyclostomes as a sucker, they have acquired another arrangement, and the water is not only discharged through the gill-slits, but is also inhaled through them, and, in the myxenoids, through the nasal passage also, which has in the tunicates become the functional mouth. The vertebrate mouth has lost its old function in the cyclo-

stome-like ancestors of the tunicates, as these have gradually lost their parasitic habit, and have established themselves on lifeless bodies; but the original lips have remained, and they are to be recognized in the so-called sucking knobs of the ascidian larva, while the teeth of the cyclostomes are supposed to be represented by "bristle-carrying end knobs" upon the suckers.

The "so-called larva" of the ascidians is represented in almost every feature of its organization by the adult, sexually mature, appendicularia. No better example of the correspondence between an adult animal and an ontogenetic stage in the history of another can be desired, and we may feel confident that, whatever the phylogenetic history of appendicularia has been, that of the ascidian larva has been the same.

Nearly all of the students who have devoted themselves to the study of the tunicates agree in regarding appendicularia as a persistent representative of their primitive condition; but appendicularia is an active swimming organism, and I have shown that its simple structure is so well adapted to the needs of its pelagic life, that there can be no inherent improbability in the view that it owes its origin to simple pelagic influences.

Nothing whatever in its habit of life or in its structure lends the least support to the view that it is a degenerated animal, and if we accept it as evidence, we are forced to believe that, far from being the fixed and degenerated descendants of parasitic vertebrates, the tunicates are descended from free, active, pelagic animals of very simple structure and minute size.

Even Dohrn seems to admit that the ancestors of the tunicates were swimming animals, for he tells us in support of his view of the homology of the endostyle (Studien, etc., VIII, p. 62) that the ancestors of the tunicates were "obviously" free swimming animals, and therefore in the position to seize their food by hunting. "Offenbar waren sie frei schwimmende Geschöpfe und damit in der Lage, ihre Nahrung durch Jagd selbst zu packen."

If the tunicates are, as their embryology and comparative anatomy indicate, the descendants of an ancestor which was *obviously* a free swimming animal, it is surely simpler, in view of all the facts, to regard the gill-slits as perforations which were originally retained and fixed by natural selection as channels for the exit of the water which was taken into the mouth with the food, than to refer them back to imaginary segmental organs which have left no other trace of their existence in the body of any known tunicata.

Minute pelagic animals, with soft bodies bathed on all sides by pure water, do not need special organs of excretion or respiration, and it is not at all probable that the pharyngeal clefts were originally respiratory; but it is easy to understand how the channels through which the water flowed became converted into gill-slits, in accordance with the law of change of function, as the descendants of the primitive tunicates grew larger and became sedentary, and thus came to need respiratory organs.

It may be argued that the thing to be explained is not the existence of gill-slits, but their serial reduplication or metamerism. It may be held that the metameric repetition of the gill-slits of ascidians forces us to regard the ascidian pharynx as the primitive form, from which that of appendicularia has been produced by "degeneration." As we are told, however, by no less an authority than Dohrn (Studien, IX, p. 417, and VIII, p. 61) that the great number of gill-slits in the ascidians is due to secondary multiplication, "nachträgliche Vermehrung," this considera-tion need not detain us.

If the logical conditions of sound morphological philosophy admit the possibility of "nachträgliche Vermehrung," and permit us to believe that the twenty or thirty pairs of gill-slits which are found in ascidians are to be traced back to the eight pairs which the primitive fishes are said to have possessed, the same logic will surely permit us to believe, on sufficient evidence, that they have arisen not from eight but from a single pair like those of appendicularia.

All the vertebrates have a peculiar organ known as the thyroid gland, and while it holds no prominent place in our general conception of a vertebrate, this gland is actually one of their most constant and characteristic organs.

In all the jawed vertebrates, from the sharks up to man, its typical structure is adhered to so closely as to prove that the gland as it exists in man is an organ of vast antiquity. In all these animals it is a ductless gland, situated far back in the throat, behind the hyoid skeleton; but at an early stage in its ontogeny it is a part of the endodermal epithelium of the pharynx, and it arises on the middle line just within the mouth.

Its function in the jawed vertebrates is problematical, but these two features in its ontogeny seem to show that far back in the remote past, before it had assumed its characteristic form, it had another function which stood in some direct relation to the mouth.

The tunicate endostyle is a conspicuous organ which attracts the eye of all observers, but its true structure was first demonstrated by Fol,

who proved that it is a pharyngeal gland with excretory cells to produce slime, and with ciliated cells to drive the slime out through the long, narrow, slit-like duct into the pharynx. Fol also showed its true relation to the ciliated peripharyngeal bands and dorsal lamella, and proved by simple but conclusive experiments that these organs are co-ordinated parts of a single system, which has for its function the capture of the microscopic floating food which enters the mouth with the water.

W. Müller was the first to point out the homology of the tunicata endostyle with the vertebrate thyroid gland, and this homology has been established beyond the possibility of doubt by Schneider's discovery that the thyroid body of ammocœtes is a slime gland with an opening into the pharynx near the mouth, and that on each side of this opening a ciliated furrow or peripharyngeal band runs upwards on the inner wall of the pharynx, just in front of the first gill-slit, to its dorsal middle line, where the two unite to form an epipharyngeal band or dorsal lamella which runs backwards to the œsophagus. Even more conclusive proof of this homology is afforded by Dohrn's account (Studien, VIII) of the histological structure of the pharyngeal gland of ammocœtes, for his studies show on the one hand a most complete fundamental identity with the very peculiar and characteristic histological structure of the tunicata endostyle, and they also, on the other hand, prove its identity with the vertebrate thyroid gland, by showing that, as development progresses, it is cut up by ingrowths of connective tissue into the isolated follicles which are so characteristic of the thyroid gland. Still further confirmation is furnished by Dohrn's discovery in the torpedo embryo (Studien, VIII, p. 60) of two endodermal grooves which run from the ventral margins of the spiracles to the ventral middle line of the pharynx, to end at the median unpaired thyroid invagination in such a way as to prove that they are rudimentary peripharyngeal grooves.

This most remarkable homology can no longer be questioned. The simplest explanation, and the one which first presents itself, is the one which Müller advances, that the common ancestor of the tunicates and of the other chordata, possessed this system of organs in the form in which we now find it in the tunicates, and that while all the jawed vertebrates have inherited the ventral pharyngeal gland, it has been turned in them to some new use, as yet undiscovered by the physiologists, and has lost its primitive connection with the pharynx and its functional relation to the mouth, and has become a ductless aggregation of follicles far back in the throat.

I have tried to show that the structure and anatomical relations of this system of organs in the tunicates are quite consistent with the view that it was originally acquired for the purpose which it now serves, the capture of food.

The simplest explanation of its origin is that which attributes it to the preservation by natural selection of a long series of slight changes, each of which improved the adaptation to the simple conditions of primitive pelagic life.

Dohrn disputes this position, and says that "many persons would have great difficulty in believing that this simple mechanism is primitive" (Studien, VIII, p. 62). The future must show how many of these persons there are, but I shall now lay before them Dohrn's own explanation, that they make comparisons for themselves.

"We ask," he says (p. 62), "how the ancestors of the tunicates obtained their food before the endostyle was formed. Obviously they were free swimming animals, and therefore in the position to seize their food by hunting. It is as certain that they needed other contrivances than the ciliated furrows and the slime-gland, as it is that the ancestors of the cirripeds sought their food in some other way than by the formation of little vortices to sweep into their mouths everything within their influence. The limbs of the swimming forefathers of the cirripeds were certainly different from the cirri of modern barnacles; even so were the ancestors of the tunicates differently constructed from the modern ones, and before the slime-gland and the ciliated grooves became the exclusive means of nutrition, they must have been the accessory aids to some more primitive mode of capturing food " . . .

"Ammocœtes lives in the sand, into which even the youngest larvæ bore. Although direct observations fail, it must be assumed that the excretion of slime and the ciliation have some advantage in the nutritive or respiratory functions of organisms which live in the mud. May we not believe that, in spite of all the sifting through the oral tentacles and the velum, the hard particles of sand would be injurious to the delicate epithelium of the gut, if this were not protected by a thick coating of slime; that the ciliated furrows are adapted for conveying this slime to the most exposed parts, and that, in this function, they have their starting-point? Once brought into existence, it is not remarkable to see these useful structures further evolved until the whole mass of food is invested with a slimy admixture to facilitate its passage through the gut. It is not impossible that besides acting mechanically as an investment, the

slime also acts chemically as an aid to digestion. If this is the case, it is easy to understand how a peculiarity so useful to sedentary animals like the ascidians, or to floating ones like the salpæ, gradually assumed the whole function of nutrition. Thus the problem of the change of function is solved."

Although it seems as if the delicate walls of the gut of a burrowing animal would be more effectively protected if slime were directly excreted "upon the most exposed spots," than by this highly specialized system of organs, we might yet believe that the system "has its starting-point" in the habits of ammocœtes, if we did not find in the structure and embryology of every chordata animal which is known to exist evidence of descent from an ancestor in which it had attained, not a starting-point merely, but its full development.

The ontogenetic evidence that the vertebrate thyroid body was at one time a pharyngeal gland opening just within the mouth, and the discovery by Dohrn of rudimentary peripharyngeal grooves in the torpedo embryo (Studien, etc., VIII, Plate H, Figs. 7f, 7g, 7h and 7i), seem to me to be convincing proofs that the organs did not have their starting-point in the habits of ammocœtes nor in any degenerated fish, but that they arose in a lineal ancestor of the selachians and of the higher vertebrates, which was also an ancestor of the tunicates and cyclostomes.

Passing now from the biological relations of the system of the endostyle to its homologies, we are told by Dohrn that it is equivalent to two pairs of gill-slits; that these gill-slits were present and functional in the fish-like ancestors of the cyclostomes and tunicates, and that two of them, the mandibular clefts, moved downwards and met on the ventral middle line to form the thyroid gland or endostyle, while the endodermal portions of the others, the spiracular clefts, lost their connection with the exterior and became converted into the peripharyngeal grooves (Studien, etc., VII and VIII).

Homologies are expressions of genetic relationship, and Dohrn tells us (p. 79) that they are valuable in phylogeny only as they furnish us with the opportunity to pass from the consideration of the structure of organs as they now exist, and of the functions of these organs at the present time, to the consideration of conditions which have passed away; to the study of the history of the modifications which have come between these structures and functions, and those which we must attribute to the same organs at an earlier genealogical stage.

I regard the structures which we find in the tunicates and in ammo-

cœtes as primitive, and as homologous with those which we find in the jawed vertebrates; and I have tried to trace the history of the modifications which have come between these structures of modern vertebrates and those which we must attribute to the same organs at an earlier genealogical stage in the primitive history of the ancestral pelagic chordata. The reader must judge of my success.

Let us now see what light Dohrn's homology throws on the history of these primitive modifications. He tells us (Studien, etc., VII, p. 47) that he will point out, further on, the significance of the changes which have led to the fusion, on the middle line, of structures which were originally paired; but I have been able to find nothing more upon this point except the acknowledgment, on page 63, that "I frankly admit that I have at present no available argument to bring the peculiar organization (of the ciliated grooves) of ammocœtes from a pair of imperforated (spiracular) gill-slits, into accordance with the concept of change of function; and that the origin of the slime-gland of ammocœtes from two ventrally fused (mandibular) gill-slits must for the present remain an unsolved problem."

Whatever may be thought of my own view, it must be admitted that Dohrn's homology of the endostylic system with two pairs of gill-slits has very little phylogenetic value, even when measured by his own test: the opportunity it furnishes for passing from the structure and functions of modern organs to the history of earlier genealogical stages.

Dohrn's memoirs upon the thyroid body are full of interesting anatomical details, such as the similarity between the thyroid body of the shark embryo and the true gill-slits, in their relations to the cartilages, to the muscles and to the blood-supply (VII, p. 44); and the resemblance between the peripharyngeal grooves of ammocœtes and the spiracular gills of selachians (VIII, p. 55); but as he admits that the annelidian hypothesis leaves the origin of the endostylic structures of tunicates an unsolved problem, our subject, the history of the tunicates, does not require us to enter into the discussion of these complicated details of vertebrate morphology.

The considerations which I have presented will undoubtedly be met by the assertion that while the simple and direct origin of the tunicates seems plausible so long as we confine ourselves to these animals alone, such a restricted view is unscientific. I shall no doubt be told that we are forced by more fundamental evidence to believe that the body cavity of the chordata is, in ultimate analysis, a segmented enterocœl formed

from a series of pairs of gut-pouches, and that the simplicity of appendi-
cularia cannot be primitive, inasmuch as the ancestors of the tunicates
once possessed these complicated structures.

The first step to take in discussing this objection is to learn whether
there are any traces of gut-pouches in the tunicates.

Seeliger (p. 9) has given us a very minute and detailed account of the
history of the mesoderm in the clavelina embryo, and has shown that it
arises from two rows of endoderm cells which give origin, in the tail, to
the caudal muscles and, in the body, to free mesoderm cells which
multiply with great rapidity and wander everywhere through the body
cavity, which is bounded on one side by the endodermal wall of the gut,
and on the other by the ectoderm.

He says emphatically (p. 128) that the mesoderm arises as two
totally unsegmented rows of cells, each forming a single layer; that the
body cavity is not an enterocœl, but a *primary body cavity;* and that the
ontogeny of the tunicate mesoderm gives no evidence of derivation from
paired pouches comparable to the cœlomic pouches of amphioxus.

It is a rare thing for students of tunicate morphology to agree, but
in this case the phenomena are simple, and Davidoff (p. 16) completely
confirms Seeliger's observations, so far as they bear upon the question,
by his own studies of clavelina and distaplia.

His account of the origin of the mesoderm differs from Seeliger's in
only one minor point, which has no bearing upon the question under
consideration. Like Seeliger, he derives the mesoderm from two rows
of endoderm cells, but he says that these cells remain as endoderm cells
after they have given rise to the mesoderm, while Seeliger states that
they become converted into the mesoderm.

In all other respects Davidoff's observations are a complete confir-
mation of Seeliger's, for he says (600) that in distaplia the mesoderm
of the caudal region persists as a solid rudiment and becomes the
muscular layer of the tail, while elsewhere it breaks up into wandering
mesenchyma cells. "It is to be particularly emphasized that in no part
of the mesoderm is *any trace of segmentation* to be discovered, and that
there is not the least indication of any cavity comparable to a myocœl.
The embryonic history of the mesoderm of distaplia cannot be referred
back in any way whatever to anything comparable to Hertwig's concep-
tion of the enterocœlomata."

Of clavelina he says (607): "There is not even a transitory division
of the mesoderm into a somatopleur and a splanchnopleur. Even where

the mesoderm is two-layered, so that a parietal and a visceral layer may be distinguished, there is no homology between these layers and the bounding walls of the cœlom of the enterocœlomata."

After reviewing all the literature on the subject, he gives as the general result of his studies the statement (p. 622) that "the body cavity of the ascidians lies between the two primary germ layers and must be regarded as a blastocœl, which would be identical with the segmentation cavity if this were not temporarily obliterated during gastrulation by the contact of the ectoderm cells and endoderm cells."

While the salpa-embryo is very complicated and unfavorable for studying this question, my own observations, which have already been described, seem to show that the body cavity of salpa is, like that of clavelina and distaplia, a primary one, fundamentally identical with the segmentation cavity, and that the mesoderm arises as free mesenchyma cells derived from the endodermal blastomeres.

The body cavity of the salpa-embryo, Plate XXXV, *15*, is identical with the space between the somatic and visceral layers of follicle cells, Plate XII, *15*, and while there is a stage in which these two layers are in contact, Plate X, Fig. 9, the follicular cavity is undoubtedly the same as the cavity shown at *15* in Plate XI, Fig. 3, and this is the same as the space which is shown in Plate X, Fig. 3, between the segmenting egg and the follicle.

In the chapter on the significance of the salpa-embryo I have given my reasons for believing that this space is homologous with the segmentation cavity of more normal tunicate embryos, and if this view be correct the body cavity of salpa is not an enterocœl but a primary body cavity or blastocœl. The mesoderm of salpa consists of free migrating cells, and the chamber of the heart is part of the body cavity, so that these cells pass through it; and while salpa is a peculiarly unfavorable subject, my observations are in complete accord with those which Seeliger and Davidoff have made under simpler and more favorable conditions.

No student of the embryology of tunicates has ever described any trace of a *series* of body cavities, and Kowalevsky, the discoverer of the cœlomic pouches of amphioxus, failed to find anything comparable to them in the tunicates, although the existence of a single pair of enterocœls has been claimed by certain observers. Van Beneden and Julin (Zool. Anzeiger, 4, 1881; Bull. Acad. Belg. (3) 7, 1884; Arch. Biol. 6, 1884) believe that the anterior portion of the body cavity of ascidians arises as a pair of gut-pouches, and that its mesoderm consists of a somatopleur

and a splanchnopleur, but Davidoff has shown by careful serial sections that this statement is probably based upon erroneous observations.

Salensky holds (17, 460) that the mesoderm of the blastoderm of pyrosoma consists of two symmetrically placed cœlomic pouches, and that pyrosoma is, therefore, to be placed among the true enterocœlomata. The space between the vertebrate blastoderm and the yolk is undoubtedly homologous with the enteron, but it is by no means certain that this is the case in pyrosoma, where the food-yolk is an independent acquisition; nor do Salensky's figures show, as clearly as we might wish, that the two cœlomic vesicles open into this space, and even if this is the case, we must remember that the pyrosoma-embryo is very aberrant, and that the structure of its body cavity may be a secondary adaptation to the presence of the yolk. Taken alone it certainly is not enough to prove, without corroboration from other sources, that the body cavity of the tunicate is an enterocœl.

The ontogeny and homology of the tunicate mesoderm have been recently discussed at very great length by Seeliger (11, pp. 85–104 and pp. 126–131), by Davidoff (16, pp. 592–628), and by Salensky (17, pp. 456–462 and pp. 468–470 and 36–46), and as those who wish can find in these papers an extended presentation of the complicated and perplexing theory (?) of the mesoderm, I have attempted to treat it very briefly.

The literature shows that there is no direct evidence whatever of the existence, at any time in the history of the tunicates, of a metameric series of cœlomic pouches, and the supposed necessity for believing that such a series existed in the primitive chordata is only another aspect of the dogma that the metamerism of the vertebrates must have been inherited from a primitive metameric ancestor.

If, as I believe, the metamerism of vertebrates is secondary, the metamerism of the mesoderm and body cavity may have resulted from the duplication of a single pair of cœlomic pouches similar to those of echinoderm larvæ, and it is quite conceivable that these may have been acquired by the ancestors of the vertebrates after the divergence of the tunicates.

If, however, future research should show that there is a pair of gut-pouches in the embryo of appendicularia, or should prove in some other way that the structures which Salensky describes are true enterocœls inherited from an ancestral tunicate, such a discovery, which is certainly among the possibilities, would be no evidence that the primitive tunicate was the degenerated descendant of an ancestor with metameric gut-pouches.

At present, however, the evidence all tends to show that the ancestors of the tunicates had no such structure, and that the presence of cœlomic vesicles in pyrosoma is an adaptation to its peculiar mode of development.

Since the critical review of the literature of the embryology of salpa will require all the space which remains, I must bring this theoretical discussion to an end, and must reserve, for publication at another time, the sections to which I have referred, on the pelagic origin of the veiled medusæ, and on the morphological significance of metamerism and other vegetative duplications in animals.

PART THREE.

A CRITICAL DISCUSSION OF MY OWN OBSERVATIONS AND THOSE OF
OTHER WRITERS, ON THE SEXUAL AND THE ASEXUAL
DEVELOPMENT OF SALPA.

CHAPTERS IX, X, XI, XII, XIII, AND XIV.

CHAPTER IX.

THE ORIGIN AND MATURATION OF THE EGG OF SALPA.

Section 1.—*The Embryonic Germ Cells.*

Many names have been proposed by various authors for the mass of cells, Plate XXI, *m, n,* and Plate XXXIV, *m, n,* and Plate XLI, Fig. 7, *n,* which runs along the hæmal region of the stolon, and gives rise to the reproductive organs of the chain-salpa; but a technical term does not seem necessary, and I shall call it the germinal mass.

It makes its appearance very early in the life of the embryo, in the form of a sharply defined, compact, subspherical mass of cells, which at first differ very slightly from the other cells of the germ layers of the body of the embryo, although they are, as we shall see, quite different from the follicle cells, which are so numerous during the embryonic stages. In Salpa pinnata, the species in which I have studied it most thoroughly, it is on the middle line of the hæmal side of the body at *n* in Plate XXXV, and between the placenta *y″* and the eleoblast *k.* It marks the point where the proliferous stolon is to be developed, and its relations to the other organs of the embryo will be understood by comparing the longitudinal section in Plate XXXV with the series of horizontal sections in Plate XIX, Figs. 1, 2, 3, 4, 5. These sections need little explanation. Figure 1 passes through the œsophagus *q,* and the intestine *p,* which, in the solitary form of Salpa pinnata, is inside the gill *o.* Figure 2 passes through the ganglion *s.* Figure 3 is through the eleoblast *k,* and the endostyle *d, d.* Figure 4 shows the endodermal tube *d′* of the stolon opening into the pharynx *c,* between the halves of the endostyle, and Fig. 5 shows this tube in cross-section at *d′,* and below it the germinal mass. A portion of this section is shown, more highly magnified, in Fig. 10, with the germinal mass marked *n.* Three successive early stages in the development of the germinal mass are shown, more highly magnified, in Plate XX, Figs. 5, 6 and 7. These are portions of median longitudinal sections, like Plate XXXV, from three embryos at three successive stages. Fig. 6 of Plate XX is the youngest stage which I

have found; Fig. 5 is a little older, and Fig. 7 is still older, and is part of the section which is figured in Plate XXXV. The details of the histological structure of the germinal mass are shown in Plate XLI, Fig. 7, which is a portion of Plate XX, Fig. 5, more highly magnified.

The youngest embryo in which I have been able to find the germinal mass was only a little older than the one shown in Plate XLI, Fig. 2, and in Plate XVIII, although I failed to find it in this embryo.

As shown by Plate XLI, Fig. 7, and by the figures on Plate XX, the granular protoplasm between the closely packed nuclei is divided up into cells, although the cell boundaries are only faintly visible here and there. It will be seen from Plate XLI, Fig. 7, that the nuclei of the germinal mass n do not differ in any marked way, except in the activity with which they multiply, from the embryonic cells of the surrounding ectoderm a, and endoderm b, although they are quite different from the follicle cells, of which one is shown at B, and also from the blood corpuscles bl, and mesenchyma cells A. As Figs. 5 and 6 of Plate XX show, the eleoblast of the embryo consists, for the most part, of two sorts of cells, small, granular, amœboid blood corpuscles and mesenchyma cells, and large, vacuolated, migratory, follicle cells, in process of degeneration. These cells are all so different from those which make up the germinal mass, that there is a strong presumption against its derivation from either of them, while the resemblance between its cells and those of the ectoderm and endoderm, and the activity with which they multiply, are reasons for believing that it arises from blastomeres, like those which give rise to the germ layers of the embryo, and possibly from the division of a single blastomere. The most noteworthy peculiarities of the germinal mass at the stage shown in Plate XLI, Fig. 7, are, first, that it is homogeneous, or without differentiation among its cells, and secondly, that cells in all parts of it are multiplying by karyokinesis.

The germinal mass is present as a definite, distinctly limited structure before the stolon is formed, but as this becomes developed, the germinal mass becomes folded into it, as is shown in the figures, and it also becomes elongated into a rod which runs along the hæmal side of the stolon, as shown at n in Plate XLI, Figs. 4 and 6, and in longitudinal section in Plate XVI, Fig. 5, and in transverse section of a young stolon in Plate XXI; and also in Plate XXXIV, Fig. 1, which is from the proximal end or root of a fully grown stolon.

As it lengthens its cells become differentiated and specialized in a manner which will soon be described, and all recent writers agree that it

ultimately gives rise to the reproductive organs of the chain-salpa. My own observations show that, in Salpa pinnata at least, it gives rise to these organs and to nothing else, and that it is set apart very early in embryonic life as the embryonic germ, or, as Huxley has termed it, the generative blastema. In the discussion of the literature at the end of this section I shall refer more at length to the fact that recent writers have believed that other structures of the chain-salpa, besides the reproductive organs, are derived from it, but I must dispute this, as far at least as Salpa pinnata is concerned, for my own studies show that in this species it gives rise to the *testes*, the *eggs* with their *follicles*, and to the *fertilizing ducts*, and to nothing more.

I have not been able to trace its origin further back than the stage of Fig. 6, Plate XXI, where it is sharply defined; and an examination of Plate XVIII, and of the more highly magnified sections in Plates XVI and XVII, will show what an unfavorable subject salpa is for attempting to trace relations between the blastomeres of the segmenting egg and the germ cells.

At the earliest stage in which I have found them, Plate XX, Fig. 6, they form a compact, subspherical, granular mass, which is sharply limited on all sides by a well-defined outline, and is closely packed with transparent vesicular nuclei imbedded in a mass of granular protoplasm with faintly-marked cell outlines.

Each nucleus contains a central group of chromatin granules, and the nuclei themselves closely resemble the embryonic cells of the surrounding tissues of the body in size as well as in other respects, although they are quite different from most of the cells of the eleoblast, which are not blastodermic but accessory in their origin, and I have observed nothing which is inconsistent with the natural hypothesis that the germinal cells arise, as they do in other animals, from certain blastomeres which are derived from the fertilized egg, and that they are in all probability the products of the division of a single blastomere, although the subject belongs properly in the section on the embryology of salpa.

According to Todarro, the germinal mass is derived from a single large cell, the "primo germoblasto," which migrates from the eleoblast or "glandula germativa," into the young stolon, where it divides and gives rise to a mass of cells, the "cumulo cellulare primativo," which gives rise not only to the germinal mass, but to all the organs of the bodies of the chain-salpæ as well.

The germinal mass is not formed in the stolon, for, as we have seen,

it is present in the embryo before the stolon itself is formed, nor does it give rise to any part of the chain-salpæ except their reproductive organs, although it is easy to understand the nature of the large cell which Todarro has seen and mistaken for the " primo germoblastico."

In the lower part of the body cavity in Plate XVII, Fig. 3, which is from a series of sections from an embryo just a little younger than those in which I found the first traces of the stolon, there is an enormous cell, so large that it runs through a number of sections. It agrees closely, both in its position and in its general appearance, with the cell which he figures and describes as a " primo germoblastico," but the study of other embryos at the same stage shows that its presence at this spot at this time is purely accidental. Similar cells are found in other parts of the body at this and other stages of development, but their position is not constant, and careful study shows beyond question that they are migratory follicle cells from the roof of the placenta. They are shown in the figures of Plate XVIII in the act of migrating into the body cavity from the roof of the placenta where they are formed and set free in great numbers, especially in older embryos. As they break down and disappear, only a few are found at one time free in the body cavity, but they wander to all parts of it, and there is nothing constant in their position or number. I have never seen one inside of a stolon, but as its cavity is in free communication with the body cavity of the embryo, there is no reason why they should not sometimes be found there, and Todarro's figures show that this is what he has seen.

In my account of these migrating placenta cells I shall show that they are not derived from the egg-cell, that their function is nutritive, and that they take no part in the construction of the embryo.

I am therefore prepared to state with perfect confidence that the germinal mass is not derived from one of them.

According to Seeliger's account (11, p. 17), which will be examined soon, the amœboid mesenchyma cells of the nucleus wander from it into the cavity of the young stolon and there build up, not only the germinal mass, but the nervous system, perithoracic tubes, and other parts of the chain-salpæ. Salpa pinnata has no true nucleus, and while it may be possible that, in its origin, the germinal mass is an aggregation of amœboid blood-corpuscles from the eleoblast, there is no evidence that this is its history.

At any rate it is not built up from cells which wander into the stolon, for it is present and well defined before the stolon itself is formed. In

Plate XX, Figs. 5, 6 and 9, the ectoderm and endoderm of the stolon are represented by a' and d' respectively. In the youngest of these stages, Fig. 6, the thickened columnar ectoderm, a', of the region of the stolon barely reaches to the edge of the germinal mass n, and this does not lie in the stolon, but in the body cavity of the embryo.

The series from which this figure was drawn was complete, and the section is squarely in the middle of the region of the stolon, and shows the greatest development which it has reached at this stage.

In the next older stage, Fig. 5, the thickened ectoderm, a', reaches over something more than half of the germinal mass, and this is gradually brought into the cavity of the stolon, not by migration, but by the extension of the fold of the ectoderm until, at the stage of Fig. 7, it is shut into the stolon between the endoderm d' and the ectoderm a'.

In Plate XVI, Fig. 5, the fold of ectoderm has run back beyond the germinal mass, and this is now in the cavity of the stolon, as is shown by the transverse sections in Plate XX, but the process by which it reaches this position is *infolding* and not *migration,* and the figures show that it is set apart as a definitely specialized group of cells before it is shut into the stolon.

SECTION 2.—*Seeliger's Account of the Germinal Mass.*

The most extensive account of the germinal mass is the one given by Seeliger (p. 11), and so far as the later stages in its history are concerned, my work is no more than a verification of his, but as regards its origin my observations are totally irreconcilable with his.

I am confident that this disagreement is due to the fact that the species which I have studied most thoroughly, Salpa pinnata, is exceptionally favorable, while the one Seeliger studied is peculiarly unfavorable for observations upon the early stages in the history of the stolon. This is symmetrically placed in the embryo of Salpa pinnata, and sections may be made in any desired plane, even when it is too small to isolate. As it is perfectly straight, any section in a series may be directly compared with any other without difficulty. Furthermore, all its parts are fully developed without any crowding, as the absence of a twist prevents one-sided pressure.

In Salpa democratica, the twisting of the stolon begins at a very early stage, and twists all its parts out of the plane of symmetry in such a way that no two consecutive sections are morphologically parallel to

each other, and it is a very small object, while the stolon of Salpa pinnata is much larger. I have found that even with the insight which I had gained from the study of the straight stolons of Salpa pinnata and Salpa cylindrica, the twisted stolons of Salpa cordiformis and Salpa democratica are very puzzling, and while I am thus in the position to recognize the industry which Seeliger has displayed, and the faithfulness with which he has tried to overcome the difficulties, I am sure that the more favorable nature of my material has enabled me to reach more trustworthy results.

The first point of difference is the time when the germinal mass becomes differentiated from the other structure.

The young stolon, Plate XXI, Fig. 7, contains a central tube of endoderm, d', within an outer tube of ectoderm, a'. Seeliger says (page 16) that at a very early stage all the space between these two tubes, that is, all the body cavity of the stolon, is filled by an undifferentiated mass of mesoderm, which soon divides into a ventral genital string, n of my figures, a dorsal nerve tube, t, and two lateral side strings, g and h.

I find, however, that the germinal mass is sharply separated, at all stages, from the other organs of the stolon, as will be seen by comparing the figures in Plate XXI with the younger stage shown in Plate XX, Figs. 1, 2, 3 and 7. At a still younger stage, Plate XX, Fig. 6, the germinal mass, which is shown more highly magnified in Plate XLI, Fig. 7, lies in the body cavity of the embryo, and, before the tubes of the stolon are formed, it is sharply limited by a distinct boundary, which is not lost at any subsequent stage.

Instead of arising, as Seeliger says, within the stolon, it is present before the stolon is formed, and it has nothing in common with the nerve tube or the lateral tubes, as I shall show in the chapter on the stolon.

I have studied sections of each stage in the three rectangular planes of symmetry, and I appeal, with all confidence, to future observers for the confirmation of my statement.

As regards its origin also, I must take issue with Seeliger, who says that the "mesoderm" of the stolon is formed by free cells which migrate into it, as independent cells, from the body cavity of the embryo.

As shown in Plate XVII, Fig. 3, and Plate XVIII, Fig. 6, and also in Plate XX, Figs. 5 and 6, the body cavity of the embryo is filled by a complicated and confused mass of cells, among which two types predominate. These are small, amœboid blood corpuscles, or mesenchyma cells, Plate XLI, Fig. 7, A, and the migratory follicle cells B.

Seeliger is disposed to regard the mesenchyma cells as the source of the "mesoderm" of the stolon, but the very small size of their nuclei as compared with those of the germinal mass renders this very doubtful. In fact an entire mesenchyma cell is hardly as large as one of the germinal nuclei, as will be seen from the figures. The nuclei of the migratory follicle cells, Plate XLI, Fig. 7, *B*, are about equal in size to those of the germinal cells, but they are vesicular and transparent, with only a few minute granules of chromatin, and they multiply directly, so that each follicle cell usually contains two, as is shown in the figure, and also in Plate XX, Figs. 5 and 6; while the germinal nuclei contain large chromatin granules, and they multiply by indirect division with nuclear figures.

I have not been able to trace the actual connection between any of the blastomeres of the embryo and the germ cells, but in the chapter on the germ layers I shall show that the blastomeres are distinguished by features which are found again in the cells of the germinal mass, and there is therefore reason to believe that they are the source from which these latter are derived.

Plate XX, Fig. 6, shows the youngest germinal mass which I have found, and another section from the same specimen is shown, much more magnified, in Plate XLI, Fig. 7. In both figures *f* is the pericardium, *c* is the pharynx, *d'* is the endoderm, and *a'* the ectoderm. The germinal mass is a granular, protoblastic body, with faintly-marked cell outlines filled with transparent nuclei a little larger than those of the endoderm and a little smaller than those of the ectoderm. The nucleus has a membrane, and it contains granules of considerable size, which are usually placed around the periphery in such a way as to appear like a dotted or broken outline.

Cell multiplication goes on rapidly at this stage in all parts of the germinal mass, and two or three nuclear figures may be found in nearly every one of the sections.

Seeliger believes that his observations on pyrosoma (15) confirm his account of the origin of the germinal mass in salpa, and he says that the space between the ectoderm and the endoderm of the young stolon becomes filled in pyrosoma, as he has described it in salpa, by an unspecialized mass of migratory mesoderm cells, from some of which the genital rod is afterwards differentiated, while the rest of them give rise to the nerve-tube and perithoracic tubes and the mesoderm.

Salensky, however, who has recently made a minute study of the

early stages in the formation of the stolon of the primary ascidiozooid of pyrosoma (17, pp. 50–56), says that Seeliger has failed to find the first stages, and he himself gives an account of the origin of the germinal mass which agrees with my own observations on salpa in all the points of disagreement between my own account and Seeliger's account of its origin in pyrosoma and salpa, for he shows that it can be recognized as a definite mass of cells before the stolon itself is formed ; that neither the nerve-tube nor the perithoracic tubes are derived from it, and that it gives rise to the reproductive organs and to them alone. He says (page 52) : "Long before the stolon begins to project, the group of fixed mesenchyma cells which forms the first rudiment of the genital rod makes its appearance on the lower wall of the subintestinal blood sinus. Thus the point where the stolon is afterwards produced is designated. Neither in surface-views nor in sections can we find, at this time, any projection from the outer wall of the body of the ascidiozooid to indicate the existence of a stolon."

Section 3.—*The Differentiation of the Germinal Cells.*

At first the germinal mass is homogeneous, Plate XX, Fig. 6, and consists throughout of embryonic cells which are uniform in character and which do not present any marked difference from the cells of blastodermic origin in other parts of the embryo. Soon differences appear among them, and as the stolon grows and the germinal mass becomes elongated, the amount of differentiation becomes greatest at the extremity, as shown in Plate XVI, Fig. 5; while the proximal end retains its embryonic, undifferentiated character. The changes may therefore be studied either in a series of embryos, or in a series of sections through different parts of a stolon which is somewhat advanced in development.

I have employed both methods, and I give figures of sections from a series of stolons, and also a series of successive stages from base to tip of each stolon.

Plate XX, Figs. 5, 7 and 6, are longitudinal vertical sections through very young stolons. Plate XVI, Fig. 5, is a longitudinal section of an older stolon. Plate XX, Figs. 1, 2, 3 and 4, are transverse sections through a stolon a little older than the one shown in Fig. 7 ; from an embryo like the one shown in Plate XLI, Fig. 3. Plate XVI, Fig. 5, is a longitudinal section of a stage somewhat older, and the figures on Plate XXI are successive sections, from base to tip, of a stolon from an embryo like the one shown in Plate XLI, Fig. 5.

Plate XVI, Fig. 4, is a transverse section near the middle of the stolon from an older embryo, in which the bodies of the chain-salpæ are indicated at the extreme tip by constrictions. Plate XXXIV, Fig. 1, is a transverse section through the base of a stolon from a fully grown Salpa pinnata, which carried a long series of buds and had set free a great number of chain-salpæ. Plate XXXIV, Fig. 2, is a more enlarged transverse section, and Fig. 4 a horizontal section of its germinal mass. Plate XV, Fig. 2, is a similar horizontal section at a short distance from the base of the same stolon, and other horizontal sections, farther and farther from the base, are shown in Plate XXII, Figs. 1 and 2, Plate XXIII, Figs. 1, 2 and 5, Plate XXVII, Figs. 1, *A*, *B*, and *C*, *m*, Plate XXVI, Fig. 3, *A*, *B*, and *C*, *m* and *n*, Fig. 2, *E* and *F*, *m* and *n*, Plate XXV, *F*, *G*, and *H*, and so on.

The first change which takes place is the differentiation of the peripheral cells from the central ones, as shown in Plate XX, Fig. 1 and Fig. 6. As is shown in the series of figures above enumerated, the peripheral cells gradually assume the character of an epithelium, and this change first takes place at the distal end on the hæmal surface, and gradually runs backwards and also spreads upwards over the sides of the germinal mass, until at last the central core of cells, *n*, is shut in by a follicular epithelium, *m*, as shown in Plate XVI, Fig. 5. In all except very young specimens, the epithelium on the hæmal side of the germinal mass extends from end to end, but for a long time the epithelium is absent from the dorsal side, and, even in old stolons, there is an unspecialized remnant of the embryonic germinal mass at the root of the stolon.

A series of transverse sections across the genital string is shown highly magnified in Plate XXXI, Figs. 4, 5, 6, 7, 8 and 9. They are from an embryo of about the same age as the one shown in Plate XLI, Fig. 5, and the entire stolon is shown in surface-view in Plate XLI, Fig. 6. The stolon is completely formed, but there are as yet no traces of the constrictions to mark out the bodies of the chain-salpæ, although these make their appearance at about this time and are sometimes found at the tips of much younger stolons.

Near the base of the stolon, Fig. 4, the greater part of the genital string consists of embryonic germ cells, and only those upon the hæmal surface nearest the ectodermal wall of the stolon are arranged in an epithelium. On the middle line the epithelium is well marked, and it consists of cells which are quite different from those which make up the rest of the genital string, but at the sides it gradually loses its distinct

character and becomes merged, without any abrupt boundary, in the germinal mass. The epithelial cells are elongated, with well-defined outlines, and the nuclei, which are a little smaller than those of the germ cells, are finely granular, and more deeply stained than those of the latter cells. When about to divide the nucleus becomes very large, vesicular, with its chromatin gathered at the center, and these large nuclei protrude from the plane of the others. All the rest of the genital string consists, in this section, of a mass of embryonic cells, crowded together and with a very thin layer of protoplasm between them, around the transparent vesicular nuclei, each of which contains a few large granules. The nuclei are a little larger than those of the epithelium, but not very different in size from those of the surrounding tissues. There are about 30 or 40 of them in each cross-section of this part of the stolon at this stage, but it is difficult to count them, as many of those which are shown in one section actually belong to the planes of other sections, and are only grazed by the cut.

Fig. 5 of Plate XXXI is a section through the germinal mass of the same stolon, a little farther away from the root, and Figs. 6, 7 and 8 are successively farther away, and the last, Fig. 8, is near the tip of the stolon. Fig. 9 is from a stolon a little older, and not quite so near the tip. It will be seen from these figures, and from those in Plate XXI, that the follicle covers all of the germinal mass, except a little of the embryonic portion at the root of the stolon, and it will also be seen that the cells which remain inside very quickly assume the characteristics of eggs, and undergo no change, except a gradual increase in size, and in the amount and distribution of the chromatin in the nucleus, and in its rapid growth, until the eggs are fertilized. In the embryonic germinal mass, Plate XVI, Fig. 7, and at the proximal end of one from a mature stolon, Fig. 8, all the nuclei are capable of multiplication, and their chromatin is scanty and consists of scattered granules, but while the peripheral cells retain their power to multiply, the central cells lose it completely as soon as the differentiation takes place, and from this time on they are definitive ova. Their chromatin gradually takes the shape of a network with a large central nucleolus, as is shown, from the base of an old stolon, in Plate XLI, Fig. 9, and from the distal end of a young one, in the figures on Plate XXXI.

The follicle ultimately gives rise, as I have shown, to the testes of the chain-salpæ, and to the egg capsules and the fertilizing ducts, which need not be further traced here, and also to migrating follicle cells, which pass

in among the eggs and nourish them, as will be described in the section on the maturation of the eggs.

Section 4.—*The Homology of the Germinal Mass.*

As soon as the germinal mass has become differentiated into a superficial layer of cells and a central core, the central cells lose the power of vegetative multiplication, and thus become set apart as eggs, as I shall show more in detail in the next section.

While they continue to grow and mature, they undergo no vegetative change until they have been fertilized, and as the differentiation takes place long before the bodies of the chain-salpæ are formed, there does not seem to be any escape from the admission that the egg, as a definite independent cell, is older than the body of the chain-salpa which carries it, and that the central cells are true eggs at the stages shown in Plate XLI, Fig. 9, Plate XXI, and Plate XXXIV, Figs. 2 and 4, and in Seeliger's Plate IV, Figs. 1, 2, 3, 4 and 5.

We must not permit this truth to be obscured by the fact that, at an earlier stage, the embryonic, undifferentiated germinal mass, Plate XLI, Fig. 7, unquestionably contains the potency of many tissues besides the eggs. We cannot, at this early stage, term it the *ovary*, although my studies of Salpa pinnata show that all the structures which are derived from it are in one way or another concerned in reproduction. It gives rise on the one hand to the essential reproductive elements, the ova, and the cells of the testes of the chain-salpæ, and on the other hand to the follicle and the fertilizing duct, structures which, while necessary to reproduction, are out of the line of genetic succession.

The nature and homology of the undifferentiated germinal mass has caused much discussion, and while this has no bearing upon the subsequent, although very early, differentiation of the eggs, the questions to which it has given rise are both interesting and important.

Two views of its nature are possible, and both have advocates.

We may on the one hand regard it as part of the soma, as a constituent part of the body of the embryo, destined to become differentiated into its organs, or those of the animals formed from it by budding. On the other hand we may regard it as a body of cells set apart for reproduction and secondarily differentiated into essential and accessory reproductive cells.

Assuming the integrity and continuity of germinal plasma, the first

view implies that the germinal mass is partly somatic in its properties and partly germinal, since it gives rise to both germ cells and somatic structures. According to the second view it is entirely germinal, and is, so far as its remote origin is concerned, directly derived from the ancestral germ cells of the primitive metazoon, although in comparatively recent times it has become differentiated into two portions, of which one retains its phylogenetic significance, and gives rise to germ cells, while the other produces structures which are only indirectly concerned in sexual reproduction. According to this view, the phylogenetic history of the follicle and the fertilizing duct is different from that of the other organs of the body, which have been represented at each step in the long series of ancestral forms by corresponding somatic structures, while these organs have no remote somatic representatives, since they have been recently evolved as the result of differentiation among the germ cells themselves.

According to the first view, the follicle cells are no more closely related than ganglion cells or muscle cells to the germ cells, while according to the second view they are homologous with, and were recently equal to or identical with germ cells.

In a certain sense every cell of the body is homologous with a germ cell, and the difference between the two views concerns the antiquity and completeness of the differentiation, and the time when it is established in the ontogenetic development of the embryo.

Seeliger is an advocate of the first view. He says (11, p. 42): " I believe that the young cells of the ovary, which have the power to produce by fission or budding other cells which become converted into the follicle, are not to be regarded as eggs, nor held to be homologous with the primitive, phylogenetic, unicellular stage to which the whole animal kingdom is to be traced back. The production of the follicle is therefore not the beginning, but the last stage of ontogenetic development ; the last change which takes place in the body of the metazoon, when it is almost complete, and before it gives an independent existence to the egg cell, in which the species reverts to the primitive stem form." He advances this as a hypothetical explanation of the alleged formation of test cells and follicle cells from ova, although he does not give assent to the statement that this actually takes place.

Cell multiplication goes on with energy in the embryonic, undifferentiated, germinal mass of the salpa embryo, as well as in the corresponding embryonic region at the root of the stolon in mature salpæ, and

some of the cells which are thus formed become ova, and others follicle cells.

Seeliger says (11, p. 36) that he could not determine whether the cells which are to become eggs are different from the others from the first or not, but he thinks that this is not the case, and that it is their position alone which determines that the central cells shall become eggs. I cannot find the slightest difference between the peripheral cells and the central ones before the differentiation takes place, and agree with Seeliger that the part which falls to each cell at the time when the differentiation takes place is determined by its position, although I believe that all are potentially germ cells.

I have never found any trace of multiplication in cells which have become definite ova, until they produce true blastomeres by segmentation after fertilization, and salpa gives no support to the view that the follicle cells are true definitive eggs at any stage of their ontogenetic history, and I fully agree with Seeliger (11, p. 37) that there is no evidence that the true eggs of salpa can revert to their embryonic condition or become converted into any other sort of cells capable of continuous life.

Salensky seems to hold the same opinion, for he says (17, p. 80), "I shall not here discuss the question of the origin of the test cells, since this can be settled only by special investigation; so far as salpa and pyrosoma are concerned, it may be stated with confidence that the calymmocytes are extra ovular in origin and are simply migrating follicle cells."

There are no "test cells" as distinguished from follicle cells, and these latter are certainly not derived from definitive eggs. The discussion of the interminable "test cell" literature is unprofitable, but Seeliger says that if we grant that in certain tunicates they are derived from the egg, this would seem to indicate simply that the cell in question is not a true egg, but rather an embryonic cell, consisting, in part, of somatic material (11, p. 43).

Salensky unhesitatingly adopts a view directly opposed to that of Seeliger, and holds that the follicle is not part of the soma, but part of the germ, and he says (17, p. 81) that the fundamental identity of the egg cells and follicle cells is so evident that it is not necessary to dwell upon it. He says that the correctness of this view is proved by the transformation of true egg cells into follicle cells, and also by the fact that in salpa and pyrosoma the follicle cells contribute, like ordinary blastomeres,

to the construction of the embryo. So far as salpa is concerned, I assert even more confidently than Seeliger that no definitive eggs ever give rise directly, before fertilization, to follicle cells, and while certain of the eggs of Salpa democratica unquestionably become abortive, there is no reason to think they continue to live in another form or become converted into other cells. I have shown in the chapter on embryology of Salpa pinnata that this species, at least, conforms to the analogy of the rest of the animal kingdom, and that its germ layers are derived from the blastomeres of the fertilized egg, while the follicle cells take no part, except a nutritive one, in the formation of the embryo, and lose their identity as living cells before they contribute to its growth. They help to form the body, it is true, but they have no more to do with its cellular constitution than the radiolarians which pass into its stomach.

While I am forced to oppose, *in toto*, Salensky's view of the fate of the follicle cells, and am therefore unable to attach any weight to his argument on this basis in favor of the homology of the follicle cells, I am nevertheless disposed to accept his homology rather than Seeliger's, and to regard the epithelial cells of the ovary and their derivatives, the follicle and fertilizing duct, as homologous with germ cells.

This view presents no inherent difficulty. The yolk gland of a cestode or of a turbellarian unquestionably consists of cells homologous with eggs, and there can be no doubt that if we could trace it back to its origin in remote ancestors we should find its equivalent, not in any organ of the body, but in germ cells. Specialization of ovarian ova for some other purpose than sexual reproduction is not unusual, and the gasteropod molluscs and the insects present many examples. In most cases the function is a nutritive one, and as I shall show that the function of the follicle cells of salpa is exclusively nutritive, there is abundant analogy for the view that they are homologous with eggs, nor is there any inherent difficulty in believing that an accessory reproductive organ, like the fertilizing duct, may have had a similar origin.

Whether this is the case or not is a question of phylogeny to be determined by the methods of comparative anatomy, and Salensky, p. 75, appeals to Bolles Lee's account of the reproductive organs of appendicularia and to the studies of the reproductive organs of ascidians by E. van Beneden and Julin in support of his position, and certainly the weight of evidence seems to favor the view that all the cells of the germinal mass are germ cells, although it must be confessed that the subject is hypothetical and that there is little basis for a decided opinion.

Uljanin holds the same view, and says (7, p. 42) that the mass of cells from which the eggs of doliolum, as well as the follicle around them, are derived, must be regarded as corresponding to the germinal epithelium of vertebrates. A few of the cells become eggs, as in the elasmobranchs and other vertebrates, and these are nourished at the expense of the other cells in their vicinity, a few of which give rise, later on, to the follicle which surrounds the eggs.

Seeliger, in his account of the origin of the eggs of clavelina (p. 6), seems to hold the same view, and says that only a few of the cells which make up the rudiment of the ovary become eggs, while the others are arrested and turned to nutritive purposes, and that it may well be the position of each cell in the young ovary and its relations to the sources of nutrition which determine what its fate shall be. He speaks of the arrested cells as *sisters* of those which become eggs, and in the case of clavelina he clearly regards all the cells as homologous with each other, and as potential eggs.

Van Beneden and Julin (Rech. sur la morphologie des Tuniciers, Arch. Biol., 1884, pp. 350–3) hold that the primordial ova and the cells of the follicle are homologous structures, arising by divergent specialization in the germinal epithelium.

Davidoff (16, p. 124) says that his study of the origin and nature of the follicle cells in distaplia and clavelina has led him to results which are in perfect accord with those of the last authors. It is hardly necessary to state that, in other groups than the tunicates, the follicle cells are almost universally regarded as germ cells which have been secondarily set apart for other functions.

In the section on the follicle I shall have occasion to refer to the subject again, and to quote observations in other groups of the animal kingdom, but those to which I have referred are enough to show that prevailing opinion is on the side of Salensky as opposed to Seeliger; and Salensky's view that the follicle cells are potentially germ cells, and that their forerunners in the bodies of distant ancestors were actual germ cells, seems to me the most logical position.

SECTION 5.—*The Maturation of the Egg.*

In Plate XXXI, Figs. 4, 5, 6, 7 and 8, are given five sections through the germinal mass, from a stolon a little older than the one shown in Plate XXI, and in about the same stage as Plate XLI, Fig. 6. They are

camera drawings, with a Bausch and Lomb one-eighth objective, and are reduced one-half in photolithography. The stolon exhibited no traces of constrictions, although Fig. 9 is from the tip of a stolon a little older, with faint indications of two or three constrictions at its tip. In Fig. 4, from the root of the stolon, the greater part of the germinal mass consists of embryonic cells like those shown in Plate XLI, Fig. 7, although the cells on the hæmal side are arranged in a distinct epithelial layer, which passes gradually at the sides into the undifferentiated mass without any sharply defined limit. An orange color has been used for the undifferentiated cells and blue for the epithelium, and the contrast of these colors gives to the epithelium in the drawings a sharpness which is unnatural. In Fig. 5 the epithelium has almost, and in Figs. 6, 7 and 8 it has quite surrounded the central cells, which, from this time on, are conspicuously different from those of the epithelium. The nucleus is much larger, and it has a large, conspicuous nucleolus, which is suspended near the center of the nucleus by a network of fine threads, which all unite again in a granular layer around the periphery of the nucleus. These structures all stain intensely, while the remainder of the nucleus remains clear and colorless, without any traces of the staining fluid. The nuclei are imbedded in a granular yolk, in which the cell boundaries are unrecognizable, although they were faintly indicated at the stage shown in Fig. 5 and in Plate XLI, Fig. 7. As we pass from the base of the stolon to the tip, Figs. 5, 6, 7 and 8, the yolk grows more and more abundant, the nuclei grow larger, and the threads of chromatin become larger and more numerous, the nucleolus especially increasing in size and in the definiteness of its outline. Plate XXXIV, Fig. 4, is a longitudinal section, or a section at right angles to Plate XXXI, Fig. 8, in nearly the same part of the germinal mass, although the latter figure is from the tip of a young stolon, while Plate XXXIV is from the base or root of an old one. At about the stage of development shown in these figures, the outlines of the eggs become much more distinct than they were at earlier stages, and the eggs have now attained to nearly or quite their full size. It is important to note that this condition is reached both in the young stolon, Plate XXXI, Fig. 8, and also at the young end of the mature stolon, before any traces appear of the bodies of the chain-salpæ. At the stage of Plate XXXIV, Fig. 1, the stolon is a continuous tube, but at the stage of Figs. 4, 5 and 6 we find the nerve-tube *t*, the perithoracic tubes *w*, and the digestive tube *d'*, continuous, while the bodies of the salpæ are indicated only by the undulating outline of the ectoderm, Figs.

5 and 6, *a*. The history of the egg from this stage to the time when it is fertilized will be understood by the examination of the successive stages shown in the following series of figures, XXII, Figs. 1 and 2; XXIII, Figs. 1, 2 and 5; XXVI, and XXVII, *A*, *B*, and *C*; XXV and XXVI, *D*, *E*, and *F*; XXV, *G* and *H*; XXXIII, *I*, *J*, and *K*; XXXII, *L*, *M*, and *N*.

The study of this series shows that, after the stage shown in Plate XXXI, Fig. 5, the only change in these cells is the growth of the yolk and the nucleus, and an increase in the amount of chromatin in the nucleus. Beyond question they must be regarded as eggs at all stages after they have become differentiated from the peripheral cells of the germinal mass; and while the cells of the embryonic germinal mass are often found in all stages of cell multiplication, my own observations confirm Seeliger's statement that, after the central cells have become set apart from the others they lose this power, and exhibit no indications of multiplication until they have been fertilized. I do not see how there can be any escape from the admission that the central cells of Fig. 5 and all the later stages are eggs, except by questioning the accuracy of my observations.

The existence of a cavity between the eggs and the follicle has been affirmed by some writers and denied by others, but it does not seem to be a point of any importance. The inner ends of the follicle cells are sharply defined, and in some specimens I have found them separated, by an empty space, from the yolk, as is shown in the figures in Plate XXXI. In other specimens, however, no such space was found, and the yolk was in close contact with the epithelium on all sides.

The source of material for the growth of the egg cells is a more important question. The reproductive organ lies in the ventral blood space of the stolon, and as the follicle cells are in direct contact with the nutritive fluid, which is driven through the stolon by the heart of the solitary salpa, they are favorably placed for growth, and in nearly every section their nuclei exhibit indications of multiplication. At the stage shown in Figs. 5 and 6, and occasionally in older stolons, certain bodies of disputed origin are found among the eggs. These bodies are characterized by the intensity of their color in stained specimens, and in carmine sections they are usually of a deep and uniform red, but in carefully prepared hæmatoxylin specimens, many of them, the largest ones, are seen to contain a nucleus which is more deeply stained than the surrounding cell body, and this is irregular or amœboid in shape. Scattered through the yolk are other bodies which agree with the nuclei

in size and in their color so closely as to indicate that they are free nuclei, and among these are still smaller deeply stained granules. Seeliger, who believes that the number of embryonic eggs is very much greater than the final number, regards them as abortive eggs, degenerating and breaking up to supply material for the growth of the others, and he says on page 36 that "the greater number of the cells of the ovarian string either become abortive and unite as nutritive material with the developing egg cell, or else they form the follicle and the duct." Exact numerical computation shows, however, that in Salpa pinnata, at least, there is no such excess of ovarian eggs as he assumes.

Salensky also believes in the occurrence of superfluous eggs, although he disputes Seeliger's statement that they serve as food, as he says, Pyrosoma, p. 79, "I have never met with degenerated egg cells in my preparations of the genital string of salpa. On the contrary, I have often seen that the young egg cells, which are unable to develop farther, move towards the periphery of the ovary and arrange themselves among the follicle cells. The structure of the cells is thus modified; the nuclei grow smaller, the protoplasm becomes more susceptible to staining fluids, and their size diminishes. They become more and more like the follicle cells and lie close among them. I have often seen such cells in the hæmal part of the ovary, which ultimately becomes the oviduct, and conclude from this that the superfluous egg cells become transformed into follicle cells."

My own view of the matter is that the cells in question are not egg cells at all, but migratory follicle cells, which wander in among the eggs and degenerate to supply them with food. My reasons for this view, which appears to be the most simple and natural one, will be more intelligible after I have described the process of migration and degeneration of the follicle cells at a later stage of development, but an examination of Plate XXXI, Figs. 5 and 6, shows, 1st, that the ordinary follicle cells are in active multiplication; 2d, that in the upper part of the follicle the nuclei of certain cells exhibit evidence of degeneration, since they lose their well-defined structure and become homogeneous, and stain deeply and uniformly; 3d, similar cells are found in the yolk; 4th, deeply stained bodies, like the nuclei of these cells, are also found free in the yolk. In carmine specimens, in which both cell and nucleus are deeply stained, one of these cells might be mistaken for a degenerating egg nucleus, but in good hæmatoxylin specimens, the larger ones, which are about as large as the egg nuclei, are seen to be not nuclei

but cells, and the evidence all seems to show that they are neither degenerating eggs, as Seeliger believes, nor eggs in process of transformation into follicle cells, as Salensky holds, but migratory follicle cells, undergoing degeneration and disintegration; and I regard them as the food supply for the growing eggs.

SECTION 6.—*Summary of the History of the Ovarian Eggs of Salpa.*

The first trace of the stolon of salpa is the germinal mass, which makes its appearance in the young egg-embryo at the spot where the stolon is afterwards developed. As this is formed, the germinal mass is folded into it, and it elongates with the growth of the stolon.

It is at first a simple aggregation of unspecialized embryonic cells, multiplying by karyokinesis. While there is at first no differentiation among its constituent cells, its later history shows that it represents the *testes*, the *eggs, follicles*, and *fertilizing ducts* of the chain-salpæ.

It gives rise to nothing else; all its derivatives are concerned in reproduction, and I have already given my reasons for believing that its cells are morphologically or phylogenetically germ cells, although the cells which give rise to the follicles and ducts are not germ cells in the physiological sense.

The root or proximal end of the germinal mass permanently retains its embryonic character, and even in old stolons it is undifferentiated, and its cells multiply by karyokinesis, but the distal portion soon becomes differentiated into a peripheral layer of epithelial cells, which retain their vegetative power, and continue to multiply by karyokinesis, and a central mass of ova. As soon as the differentiation takes place, and long before the young stolon shows any trace of the chain-salpæ, the central cells lose the power of multiplication, and quickly assume the optical characteristics of young eggs. They produce no "test cells," and show no indications of cell multiplication. Their integrity as independent cells is perfectly preserved from the time of their appearance until they are fertilized in the bodies of the chain-salpæ.

They are at first immature, and they grow and ripen, but they are, obviously, true eggs, physiologically as well as morphologically, from the first. New eggs arise nowhere except at the base of the genital string, and the outline of the egg nucleus is, from the first, sharp and uninterrupted, with no trace of nuclear buds.

At first the eggs are crowded together, but as the stolon lengthens

and as the eggs grow, they stretch out into a series which, in Salpa pinnata and Salpa cylindrica at least, ultimately become a single row. As the bodies of the chain-salpæ are formed, eggs from this row are folded off and pass into their bodies; one egg for each chain-salpa in Salpa pinnata, Salpa cylindrica, and in most species, but five for each in Salpa hexagona, where each chain-salpa gives birth to five embryos. Salpa cordiformis probably resembles Salpa hexagona in this, and Salpa democratica seems to be in a transitional stage, for while this species usually gives birth to only one embryo, it seems to receive occasionally more than one egg, the extra ones degenerating.

The peripheral cells of the germinal mass form an epithelium around the central core of eggs, and this epithelium retains its vegetative power and grows throughout the whole length of the stolon by cell multiplication. As the eggs increase in size they are nourished by cells which migrate from the epithelium in among the eggs and there degenerate.

As the string of eggs lengthens the epithelium also lengthens, and it ultimately forms a follicular capsule around each egg, or, in Salpa hexagona, around each group of eggs, so that when the eggs pass into the bodies of the chain-salpæ they are enclosed in epithelial capsules or follicles. After it has passed into the body of the chain-salpa the epithelium gives rise to the testis and the fertilizing duct, but as a knowledge of the history of these structures is not needed for our purpose, the description of them may be left for the chapter on the development of the chain-salpæ.

The only derivative of the epithelium which concerns us here is the follicle. As shown in Plate X, Fig. 1, the ripe egg is enclosed in a follicle which consists of a single layer of cubical cells, with their central ends in contact with the surface of the yolk and their outer ends bounded by a basement membrane.

Section 7.—*The Number of Eggs.*

Seeliger says, page 36, that it is easy to satisfy one's self that the number of cells which pass through the first stages in the process of modification which leads to the formation of eggs is very much greater than the final number of eggs. He therefore believes that many of these ovarian eggs are destined to become abortive, and, by degeneration, to supply a part of the nutriment for those eggs which attain maturity. He gives no numerical data as a basis for this impression regarding the

relative numbers of ovarian and mature eggs, and the facts seem to me to warrant the directly opposite view, that the number of cells in the embryonic germinal mass is very much smaller than the total number of eggs which each solitary salpa produces. Even in the oldest stolon the germinal cells at the base are in active multiplication, and new ovarian ova are continually arising at its root and traveling outwards to take the places of those which have been carried away in the bodies of successive broods of chain-salpæ. We know nothing regarding the birth-rate of salpa, except that it must be very high, for in calm weather it multiplies so rapidly that the surface of the ocean quickly becomes crowded by innumerable multitudes, and when kept in captivity in an aquarium the solitary form of Salpa democratica discharges chain after chain without any evidence of exhaustion. I know of no observations upon the maximum birth-rate of any species of salpa, but the great number of buds upon the stolon is an indication that it is very great indeed. Salpa democratica usually has three or four sets of buds upon its stolon at one time, and Leuckart (p. 67) found forty in one set and sixty-five in another. Seeliger figures thirty-one in the same species on one side of a set, or sixty-two in all, and sixty is probably very near the average for the species, and multiplying this by four we get from two hundred to two hundred and fifty as the number of segments of the stolon.

I find that a single set of buds from the stolon of Salpa cylindrica contains about two hundred, so that the whole number in the stolon at one time in this species is six or eight hundred, and the stolon of Salpa pinnata usually has from two hundred to two hundred and fifty buds.

Salpa democratica gives birth to a great many chains, and our scanty knowledge indicates, so far as it goes, that the number of buds which are found in the stolon at one time is only a very small part of the total number which each solitary salpa can produce, for the process of the formation of new buds is continually going on even in the oldest stolon, and there is always a reserve of ova at the base, and I have never seen anything to indicate that the process of budding ever comes to an end through the loss of the power to bud or the exhaustion of the supply of eggs. In some species, such as Salpa hexagona, each bud contains five eggs, and we may safely assume that the germinal mass represents in itself the possibility of many thousand eggs.

I have tried to estimate the number of nuclei in the germinal mass at the stage which is shown in Plate XX, Fig. 6, and in Plate XLI, Fig. 7.

It is difficult to count accurately through the series of sections without making a series of accurate drawings, and this is hardly worth while, as a sufficiently close approximation can be made by counting, and the number of nuclei is certainly not more than five hundred, and it is probably much less.

As the peripheral nuclei give rise to the follicle and to the structures which are derived from it, only a part of these five hundred can become eggs; but the number of eggs derived from the germinal mass during the whole history of the stolon and its buds is very much greater than five hundred, and the need for the multiplication of the embryonic germ cells is clear.

In fact, the persistence of a mass of embryonic, actively multiplying cells at the base of the old stolon is in itself an indication that the number of ova is at no time equal to the future demand.

The context of Seeliger's paper shows clearly that his statement on page 36, that "es lässt sich leicht feststellen, dass eine die spätere Eizahl um ein Mehrfaches übertreffende Anzahl von Zellen die ersten Umbildungen erfährt, welche zur Entstehung des Eies fähren," refers to the very early stages of the genital string, although both this author and Salensky (Pyrosoma, p. 78) appear to have been led to this view by the fact that in Salpa democratica, the species which these authors have investigated most exhaustively, the portion of the genital string which passes into the body of the chain-salpa contains, in addition to the single perfect egg, two or three others, which are small and abortive, and soon disappear. In Salpa pinnata this is not the case, for each chain-salpa receives only a single egg closely invested by its follicle, and I believe that the abortive eggs of Salpa democratica are to be explained in another way, as a reversion to a primitive condition of this species. Each chain-salpa of Salpa hexagona and of Salpa cordiformis receives four or five perfect eggs, as is shown in Plate XLV, Figs. 6 and 7. Salpa democratica is certainly very close to Salpa cordiformis, and it is therefore probable that its recent ancestors carried several eggs each, and produced several embryos, like Salpa cordiformis; and the abortive eggs are therefore to be regarded as eggs which underwent development in the cordiformis-like ancestor. There seems to be some slight ground for the belief that one or more of these abortive eggs of Salpa democratica may occasionally complete their development and give rise to embryos after the manner of Salpa cordiformis, for Salensky, "Neue Untersuchungen," p. 382, says that he has found an egg in a chain-salpa of this species which had already produced one embryo.

CHAPTER X.

SEX IN SALPA.

Those who are familiar with the literature may justly claim that this question has been discussed enough, and I shall not dwell upon it, although I wish to review, very briefly, some of the criticisms which have been published, upon my first paper (The Development of Salpa, Bull. Mus. Comp. Zool., 1876, pp. 291–348), in which I pointed out that since the egg, which is fertilized and developed in the body of the chain-salpa, exists as a true egg in the genital rod before there are any traces of the body of the chain-salpa, this latter cannot be the mother of an egg which is older than itself.

This view has been fully discussed by several writers, and universally rejected on the following grounds:

In the year 1877, or a few months after my paper appeared, Salensky (Morph. Jahrbuch, III, pp. 549–601) published a paper on the budding of salpa, in which he affirms that the body which I had called the ovary, and which I have in this paper called the genital rod, gives rise to the cloaca and gill and branchial sac, as well as to the egg; and in a second paper published soon after (3), he says, p. 279, that since he has shown "that the ovary arises from a part of the stolon which I [Salensky] have called the endoderm, and which also represents the rudiment of the branchial sac, this is, in my opinion, enough to show that no definite rudiment for the ovary exists in the stolon, and also to disprove the view that the solitary salpa lays its eggs in the chain-salpæ."

Salensky has recently, 1891 (17, p. 78), retracted the statement that the genital rod is endodermal, and has admitted that it does not give rise to either the cloaca, the gill, or the branchial sac, so it is plain that his criticism, above quoted, has no basis.

In 1888 and 1889, Seeliger published two papers, one (11) on the budding of salpa, and another (15) on the budding of pyrosoma, in which he states that the body cavity of the young stolon, in both salpa and pyrosoma, becomes filled by a mass of embryonic, undifferentiated mesoderm, and that from this the nerve tube, the perithoracic tubes, the mesoderm and the genital rod are afterwards differentiated.

I have shown (p. 225) that the genital mass is present in the embryo of Salpa pinnata before the stolon is formed, and that it gives rise to nothing except the reproductive organs, and Salensky (17, pp. 50–57) has recently shown that this is true of pyrosoma also.

Seeliger, in his paper on the budding of salpa, gives the first correct account of the origin of the testis, and shows that this, as well as the follicle, is derived from the genital rod.

In a paper published soon after, in 1888, on the origin of the alternation of generations of salpa (13), he says, p. 403: "Brooks bases the female nature of the solitary salpa on the *occasional* occurrence of a few cells which are distinguishable as eggs in the mesoderm when it migrates into the cavity of the stolon."

I have shown, however, p. 223, that not only a few but *all* the eggs arise in the germinal mass at the root of the stolon, and that this is the only place where new eggs are formed, either in the young stolon or in the old one.

Seeliger goes on to say that "I cannot regard Brooks' conclusion as a necessary one, for the eggs of the chain-salpæ are by no means definitively perfect in the embryo, since they continue to undergo modifications until just before fertilization, like the mother cells of the testis and the somatic cells in the mesoderm of the stolon. As we must regard the testis, for example, or the branchial sac, as organs of the chain-salpæ, whether they arise from later generations of mesoderm cells, or are derived from a definite part of the embryo; so also must we regard the ovary as an organ of the chain-salpa, since its modification from the embryonic string of cells is only quantitatively different from the modifications which the testis and the branchial sac undergo."

"Since, however, the testis of the chain form arises from the same rudiment as the ovary, it is only speculation to regard the one as belonging to the chain form, and the other to the solitary form, for the same point of view would compel us to regard the solitary form as hermaphrodite and the chain form as sexless."

If it were true that the modifications of the ovary are only *quantitatively different* from those which are undergone by the rudiments of the testis and the other organs of the chain-salpa, this objection would be conclusive, but there is a most significant difference. In the young stolon the rudiments of the testes and branchial sacs grow by cell multiplication, but after an egg cell is formed in the germinal mass it completely loses its vegetative power, and remains, until fertilization, a single definite distinct ovum, as I have shown on page 222.

In support of this view I appeal to Seeliger's own figures (11), and I will ask the reader to examine the following representations of the egg of salpa: Taf. IX, Fig. 5; Taf. VIII, Figs. 2, 3, 4; Taf. VII, Figs. 14, 11, 3, 2, 1; Taf. VI; Taf. V; Taf. IV, and Taf. III, Figs. 14, 12 and 11.

It is true that the eggs continue to grow and to ripen as the stolon develops, but no new ones are formed anywhere except in the embryonic germinal mass, and as this is recognizable in the body of the embryo, page 208, before the stolon is formed, it is plain that the eggs are older, as independent definite cells, than the chain-salpæ, and I have shown, page 19, that in those species which produce four or five embryos, four or five eggs pass into the body of each chain-salpa.

When in arthropods, cestodes, molluscs or echinoderms, the ripening place of the eggs is different from their place of origin as definite cells, this latter spot is universally regarded as the ovary, and if the bodies which are shown in my Plate XXXII, Fig. 7, are eggs, so also are those shown in Plate XXVI, Plate XXIII, Plate XV, Plate XXXIV and Plate XLI, Fig. 9; while the place where the central cells of the germinal mass are found in a state of vegetative activity, Plate XLI, Figs. 8 and 7, is the true ovary.

A third criticism of my view now remains to be noticed.

Weismann (Die Entstehung die Sexualzellen bei den Hydromedusen, Jena, 1883) says, p. 294, that the view that the development of salpa is an alternation of sexual and asexual generations, can only be set aside when it is shown that the egg cells which are contained in the stolon belonged, at an earlier phyletic period, to the nurse itself; that they were formerly the cells of the ovary of the solitary salpa, and that they have in the course of evolution been pushed out into the bodies of the chain-salpæ.

I hold that there is evidence that the eggs which are fertilized in the chain-salpæ are phylogenetically the eggs of the solitary salpa.

Each zooid of pyrosoma produces buds, and these buds in their turn produce buds, and so on indefinitely. Each of them has a testis and an ovary of its own, derived from the germinal rudiment of the preceding generation, and each of them has an egg, which is fertilized and undergoes development in its body. Each of them also produces an egg, but this passes over into a new bud, with the rudiment of the ovary and testis, to mature and ripen there and to be fertilized.

Recent writers on salpa speak of the single egg of the chain-salpa as an "ovary," but as each pyrosoma has an ovary of its own capable of

producing eggs, the single egg, which is comparable to the single egg of Salpa pinnata or Salpa cylindrica or to the five eggs of Salpa hexagona, is certainly not the ovary, but a true egg which was differentiated from the ovary of the preceding generation.

This fact was pointed out many years ago by both Huxley and Kowalevsky, and Huxley (Anatomy and Development of Pyrosoma, Trans. Linn. Soc., 1860, XXIII), after showing, p. 212, that each bud carries away one fully differentiated ovum, together with part of the reproductive organ of its parent, says: "It is not a little remarkable that the first recognizable part of the new organism should be the foundation of that structure which will eventually develop into a creature distinct from it."

Seeliger, in his paper on the origin of alternation of generations in salpa (13), p. 410, denies this fact, and says that in pyrosoma "no eggs except the ones which remain in the body of the mother are recognizable before the rudiment of the reproductive organ of the mother is differentiated from the mesoderm of the future buds; and that the eggs of following generations of buds are differentiated later."

I appeal, however, to Seeliger's own figures of pyrosoma (15) published a year later, and especially to his Taf. XXXII, Fig. 22, which I have copied in cut Z.   He says, p. 652, that this figure shows " Die Region

CUT Z.
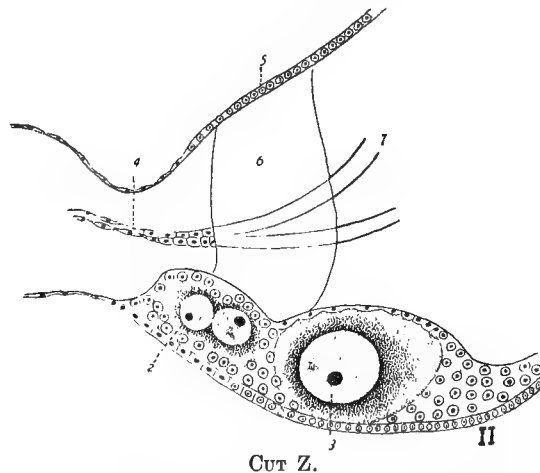
in welcher *später* der stolo prolifer zur Ausbildung gelangt," and he represents clearly, in addition to the egg marked *o* (ovum), which is destined to be fertilized in this individual, two others, marked *ms* (mesoderm), which are destined to pass "später" into the stolon in order to undergo their development within the bodies of future buds which do not yet exist.

Seeliger's conception of the nature of the process of budding forces him to regard these latter cells as "mesoderm," and I shall not discuss this subject, but shall confine myself to a request that the reader study his figures and form his opinion of the nature of these cells from them, although Seeliger himself says (p. 639) of the one I have copied, "In dem distalen Teil des Geschlechtsstränges findet man oft schon frühzeitig neben den indifferenten Zellen solche, welche durch den grossen, bläschenförmigen Kern sich als *Eizellen zu erkennen geben* (Fig. 22) und, wie die weitere Entwicklung lehrt, in der That zum Teil zu Eiern werden," and I think the question of the migration of the eggs of pyrosoma may be dismissed with this quotation.

I believe that the facts admit of only one interpretation, and prove that both salpa and pyrosoma are descended from an hermaphrodite ancestor which was very similar to the modern ascidians in structure and habits, p. 123, although it was, no doubt, more primitive than any modern ascidian, p. 128. When mature, the animal which hatched from the egg was hermaphrodite, and it gave rise, by budding, to hermaphrodite descendants which also produced hermaphrodite buds, and so on.

Whether the method of budding which is exhibited by doliolum, pyrosoma and salpa was the primitive method for all the tunicates or not, there can be no doubt that the common ancestor of these three groups gave rise on the middle line of the ventral surface to buds which were symmetrical in the middle plane of the parent, and that the digestive organs and perithoracic organs of all the buds were derived from the corresponding parts of the parent, and were therefore, in ultimate origin, part of the corresponding organs of the animal which hatched from the egg.* The hermaphrodite reproductive organs of the buds were derived in the same way from that of the first member of the series, *and were, in their ultimate origin, part of it.*

Before pyrosoma and salpa diverged from the ancestral form, each member of the series acquired the habit, so characteristic of pyrosoma, of passing eggs into the body of a bud to ripen and develop there.

At first all the individuals which make up the life-cycle were alike, both physiologically and anatomically, and there was no polymorphism.

In pyrosoma this indefinite series of similar zooids has been differentiated into a first zooid, which has become rudimentary, and is now known as the cyathozooid; a second generation of four infertile or

In a future paper I shall discuss the relation between the budding of Salpa, Pyrosoma, and Doliolum, and that of Botryllus, Botrylloides, and other ascidians.

sexually immature ascidiozooids, and an indefinite series of sexual hermaphrodite ascidizooids.

In salpa, the first generation, or solitary salpa, has enormously enlarged its capacity for budding, so that it is able to mature from fifty to two hundred buds at one time, and to produce many hundreds during its life. The second generation has lost all power to bud, so that the life-cycle has become limited to two generations.

The solitary salpa produces ovarian eggs, and, like its ancestors and like pyrosoma, passes them into the buds to mature and ripen and develop. As the aggregated salpæ do not bud, they have lost their ancestral ovaries, and all the female germ cells now remain in the body of the solitary salpa at the base of the stolon and form its ovary, *as they formed the ovary of the primitive tunicate from which the method of budding has been inherited.* The testis of the solitary salpa remains embryonic, its cells multiply by karyokinesis and grow out into the stolon, and give rise to the testes of the chain-salpæ, which, however, contain no female germ cells and produce no eggs.

Seeliger holds, indeed, that we must not compare in this way the whole life-history of salpa with the whole life-history of pyrosoma, but that we must neglect the history of the formation of the four primary ascidiozooids by budding from the body of the cyathezooid, and of the first generation of secondary ascidiozooids from the bodies of the primary ascidiozooids, and that we must compare the budding of salpa with the budding of the secondary ascidiozooids; but as the evolution of the life-history of salpa out of a dislocated fragment in the life-cycle of pyrosoma is not proved by any evidence, it is surely more rational to regard both life-histories as divergent modifications of a common ancestral life-history.

CHAPTER XI.

THE FERTILIZATION AND SEGMENTATION OF THE EGG.

SECTION 1.—*The Changes which Accompany Fertilization.*

My most complete series of very young stages is from Salpa hexagona, a species which, like Salpa cordiformis, is characterized by the presence of a series of four or five eggs or embryos. Since these are fertilized and undergo their development in succession, a number of stages in the life-history of the embryo may be obtained from each specimen, and as they are all placed in positions which are nearly alike, at least during the early stages, the species presents exceptional advantages for study. With only a limited collection of preserved specimens, sections of successive stages in planes which nearly correspond may be obtained from this species with much less difficulty than usually attends the manipulation of such minute eggs.

Unfortunately, I have had very few eggs of this species, and a still smaller number of specimens of Salpa cordiformis (zonaria).

Most of my young specimens of Salpa hexagona contained five eggs each, and I think this number is pretty constant, although this is a question which can be easily answered by any one who has an opportunity to examine an abundant supply of fresh specimens.

As is shown in Plate X, Fig. 10, and also in Plate III, Fig. 1, they form a series along the course of the so-called "oviduct," Fig. 10, X. As no eggs ever pass through this tube, it is not an oviduct in any physiological sense, and I prefer to call it the *fertilizing canal*, although it may possibly be homologous with the oviduct of other tunicates. The youngest egg, or embryo, is nearest its external opening into the atrium, and the oldest is farthest from it. In Plate X, Fig. 10, *A* is an unfertilized egg, *B* is one in which segmentation has begun, *C* is in the stage which is shown in Fig. 8, and *D* is in the stage shown in Fig. 9.

If we call that end of the fertilizing duct which opens into the cloaca proximal, there is a distinct lumen proximal to the unfertilized egg, which lies in a pouch-like enlargement or diverticulum in the wall of the duct, as is shown in cross-section in Fig. 1, where almost the whole cavity

is filled by the egg, although a small part of it runs past the egg as a tubular opening, X, to the next egg. After each egg is fertilized this tubular portion of the duct gradually becomes constricted off from the part which surrounds the egg, and this is thus completely shut in to a closed capsule or follicle. The cavity of the duct then gradually disappears, as is shown in Plate IX.

There is a conflict of opinion regarding the structure of the duct before fertilization. Barrois figures it (4) in Salpa africana, before fertilization, as a continuous tube with a lumen which runs from the external aperture to the egg. In living specimens of Salpa democratica at the same stage I have found a continuous passage, and have traced the spermatozoa through it up to the egg before the shortening begins. In his first paper (2, p. 183) Salensky figures a lumen in Salpa democratica, Fig. 4, and says: "In the preserved and colored specimens the lumen of the oviduct is not always to be discovered, but in living animals it is found with ease."

Todarro says that there is a continuous lumen in Salpa pinnata, and in my sections of Salpa hexagona it is clearly shown, as in Plate X, Fig. 10.

Figure 9 of the plate shows the way in which, *after fertilization*, its lumen gradually disappears until at last its cells become arranged in a single row.

These various observations on both living animals and sections of several species show that the act of fertilization initiates the closure and shortening of the duct; but in his second paper (5), Salensky says that in Salpa pinnata, p. 96 and p. 105; in Salpa africana, p. 147; in Salpa punctata, p. 327, and in the genus in general, p. 147, it is divided into a solid portion or egg-stalk, nearest the egg, and a distal perforated portion or oviduct with a lumen which runs only about half way from the external aperture to the egg, so that there is no open way for the spermatozoa to reach the egg. The shortening of the stalk begins, according to him, before fertilization, and takes place in such a way that the cells of the imperforated portion or egg-stalk become merged in the follicle until the egg is thus drawn along to the perforated portion to meet the spermatozoon.

That these changes take place as he describes them there is no doubt; but my observations show that they occur after fertilization, in Salpa hexagona at least, and that there is at first no solid section in the duct.

In Plate IX, Fig. 1 is the most proximal section. In this the fertilizing duct is separate from the follicle, and it has a distinct lumen. In Fig. 9 it is closely adherent to the follicle, but distinct from it, as it is throughout the whole series. In Fig. 10, the most distal section, it is separate from the follicle and solid. In an older egg, Plate X, Fig. 7, the lumen is absent, and that portion of the duct which is distal to each embryo gradually degenerates, as shown at X in Plate X, Fig. 9, and it ultimately disappears completely, while the portion of the duct immediately proximal to each embryo acquires a new opening into the atrium, as shown at *11* in Figs. 9 and 10, and becomes the suspensorium of the embryo, by means of which it is attached to the wall of the atrium, while the other end retains its connection with the follicle. Why it should acquire a new opening into the atrium I do not know, unless it is destined to take part in the formation of the fœtal membranes. These are usually regarded as folds of the wall of the atrium (or pharynx) of the chain-salpa, but it is not improbable that a more thorough study of Salpa hexagona and Salpa cordiformis may show that they are actually derived from this portion of the duct. It is certain that they are formed, in all species, from the region where the duct and the wall of the atrium are united, but I do not think the question of their origin can be regarded as settled. Salensky says that they are derived from an " Epithelhügel " or a raised thickened area, b', in Plate XI, Figs. 1 and 2, which is, according to his account, part of the wall of the pharynx, immediately around the point of attachment of the duct, but it is not at all improbable that the Epithelhügel comes from the duct itself, for if it does not, there does not seem to be any reason why, in species with several embryos, the duct should acquire a new opening for each embryo. I am therefore disposed to believe that the Epithelhügel will ultimately be shown to be a derivative of the duct, although my own material is too scanty to settle the question.

Section 2.—*Fertilization.*

A ripe unfertilized egg is shown in Plate X, Fig. 1. It lies in a blood space of the chain-salpa, close to the wall b of the atrium, and it is usually surrounded by amœboid blood corpuscles, *12*. It is enclosed in a follicle which is one cell thick, and which is simply a distended portion of the duct X. The granular yolk entirely fills the follicle except at the lumen of the duct, but this runs past this egg to the next one in the series. The large

nucleus contains a large, spherical, sharply defined nucleolus, surrounded by a granular layer which is continuous with a network of threads ending around the surface of the nucleus in a sharply defined granular border, quite different from the surrounding yolk. In the next stage which I found, Fig. 2, the egg is apparently in the act of fertilization. The yolk has shrunken away from the follicle a little, so that its own outline can be distinguished from the outline of the inner ends of the follicle cells with which it was in immediate contact at the preceding stage. At the upper end of the yolk, where the space between it and the follicle is greatest, is a deeply stained rod-like body which is probably the spermatozoon. The outline of the nucleus is vague and indefinite, the nucleolus and the network are no longer visible, and in their place near the center of the nucleus there are two faintly defined groups of minute granules. This section, Fig. 2, is like Fig. 1, transverse to the length of the fertilizing duct, and the spermatozoon is shown by the study of the series to be near the proximal surface of the egg, or that surface which is nearest the upper end of Fig. 10. It will be seen that the spermatozoon is in the middle plane of the egg, and a comparison of Figs. 1, 2 and 10 will show that as the spermatozoa pass through the fertilizing duct they must meet each egg at approximately the same point, on the middle line of the proximal surface. The follicle at this stage is quite different from Fig. 1. It is much thicker, the cell boundaries are much less sharp, and there is a great increase of follicular nuclei, and a change in their shape and general appearance. They are no longer round, but elongated in radial lines, their granules are larger and less numerous, and here and there in every section is a nucleus in some stage of division. These dividing nuclei are always near the inner border of the follicle, as shown in Figs. 2 and 3.

SECTION 3.—*Segmentation.*

Successive stages of segmentation are shown in Fig. 3 of Plate X, in Figs. 1 to 10 of Plate IX, in Fig. 11 of Plate IX, in Figs. 4 and 5 of Plate X, and in Figs. 6, 7 and 8 of Plate X.

Plate X, Fig. 3 is a section from a series through an egg which has undergone its first division, cut in a plane parallel to the oviduct, or at right angles to Figs. 1 and 2. The egg is divided in the plane of the paper into two cells; one of them with a nuclear figure which shows that it is about to divide into an upper and a lower portion. On the proximal

side of the egg, wedged in between it and the follicle, is a small cell, *13*, which is probably a pole cell.

The next egg which I found is in the eight-celled stage, and it is shown in Plate IX, Figs. 1 to 10. The sections are transverse to the oviduct and they begin on the proximal side, so that Fig. 1 is proximal and Fig. 10 distal, and the small cell with the deeply stained nucleus in the center of Fig. 1 is probably a pole cell.

It will be seen from the figures that the eight cells are symmetrically arranged in two sets of four each on the sides of a plane of bilateral symmetry, which is also the plane which, at an earlier stage, passed through the spermatozoon and the nucleus.

The eight cells are wedge-shaped, and they are crowded together in such a way that the broad ends of four of them, *1* and *2*, and the narrow ends of the others, *3* and *4*, are proximal. Two of them, the upper ones in the figures, are much smaller than the other six, which are nearly equal in size. The nuclei of all of them are in a condition of preparation for the next division, which will result in the formation of sixteen cells. Two of the cells, *3* in Figs. 6 and 7, are more advanced than the other six, and their nuclear figures show that the next division takes place parallel to the second cleavage plane.

The next stage which I have found is shown in Figs. 4 and 5 of Plate X, cut nearly, but not exactly, in the plane of Fig. 3. There are about sixteen cells, but as the planes do not coincide I was not able to trace the exact relation between them and those of the eight-celled stage.

The next stage which I have found is shown in Figs. 7 and 8. The plane of the sections is longitudinal, or essentially like Figs. 3 and 5. The blastomeres are now quite numerous, and they differ greatly in size, and as they are now separated from each other by the mass of migrating follicle cells among which they are imbedded, it is almost impossible to trace the history and fate or the exact mode of origin of each cell, at least by sections of preserved specimens, although more might possibly be made out by the observation of living eggs during segmentation.

Still older embryos are shown in Plate X, Fig. 9, and in Plate XI, Figs. 1 and 2. The blastomeres which are now scattered throughout the mass of follicle cells are not arranged in any discoverable order. Their nuclei still continue to exhibit indications of division, and the protoplasm of each blastomere now contains, besides the nucleus, a number of faintly defined bodies, which I regard as the degenerating nuclei of the follicle cells.

SECTION 4.—*The Segmentation of Salpa Hexagona as Compared with that of Clavelina.*

Seeliger has published a minute and fully illustrated account of the segmentation of the egg of clavelina, based upon observations upon living eggs, and my own somewhat scanty series of stages in the segmentation of Salpa hexagona shows that it agrees very closely with clavelina, at least during the early stages.

The first cleavage plane divides the eggs of clavelina, according to Seeliger, into a right and a left half, and each of these then divides, in a plane at right angles to the first, into two cells, one of which lies in the anterior and the other in the posterior region of the body of the future embryo. My Fig. 3, Plate X, shows one-half of an egg which has divided into two cells on the middle plane, and the nuclear figures show that each half is about to divide into an upper and a lower half, corresponding to Seeliger's anterior and posterior cells. In clavelina each of the four cells at first contains both ectodermal and endodermal plasma, but the next plane of segmentation, which is at right angles to both the first and second, results in the division of each of these cells into two, one of them dorsal and ectodermal, and the other ventral and endodermal. The eight-celled stage is bilaterally symmetrical with two ectodermal cells and two endodermal cells on each side of the middle line. In a symmetrical view of the dorsal surface the four ectodermal cells are visible; the four endodermal cells are seen in ventral view, and in side view the two ectodermal cells and the two endodermal cells of one side of the body are seen. The study of the series of sections of the eight-celled stage of Salpa hexagona shown in Plate IX, Figs. 1 to 10, will show that it agrees exactly with clavelina. It is bilaterally symmetrical with four cells on each side of the middle plane, and four also in the upper half (anterior?) and four in the lower half (posterior?); four cells have their broad ends proximal or towards the pole cell (ectodermal or dorsal?) and four are distal (endodermal?).

As shown by the nuclear figures in Figs. 6 and 7, the planes of segmentation which result in the sixteen-celled stage are parallel to the second plane of segmentation, while in clavelina, according to Seeliger, they are parallel to the first plane of segmentation. Up to the sixteen-celled stage, however, the agreement between the two is exact.

SECTION 5.—*The Significance of the Symmetry of the Salpa Embryo.*

The result of this comparison is, to my mind at least, the establishment of a strong presumption that the fertilized egg of salpa is, like that of clavelina, a potential embryo, and nothing short of the most complete and satisfactory evidence to the contrary should prevent us from believing that it is destined, in course of nature, to become an actual embryo, after the analogy of other eggs.

Seeliger's identification of the first three planes of segmentation with the regions of the body of the potential clavelina is based upon careful study, and his description inspires confidence in the accuracy and value of his conclusions ; and even if the precise relations between the cells of his eight-celled stage and the body of the embryo should prove to be somewhat different, there can be no doubt whatever that these cells and the cleavage planes which produce them out of the egg do stand in some definite relation to the potential tunicate.

The bilateral symmetry of the segmenting egg of salpa and its similarity to that of clavelina lead us to the same belief, that the eight-celled stage in salpa is a potential salpa, and that its eight cells and the planes which separate them are definite and fixed; nor is there any evidence that this regularity in the arrangement of the blastomeres is ever lost. My inability to control the planes of my sections gives a semblance of irregularity to my figures of the older stages, but there is nothing in my own sections or those figures by Salensky to show that the arrangement is actually irregular at any stage.

The segmenting egg of salpa is an embryo, beyond question ; either an embryo which normally becomes a modern salpa, or one which became an adult in the remote ancestors of the modern animals. If the latter is the case, and the body of the modern salpa arises in great part from another source, and if the blastomeres of the segmenting egg are chiefly nutritive in function rather than formative, it is difficult to understand why their regular arrangement should have been retained, for cells whose function is nutritive rather than vital are generally characterized by irregularity.

The early stages of salpa are such as to cause us to approach, in an extremely critical and exacting frame of mind, the view that, in the older stages, " Die Blastomeren mehr und mehr in den Hintergrund treten und wohl kaum irgend eine Rolle bei der Bildung der Organe spielen " (5, p. 390).

The bilateral symmetry of the salpa embryo is interesting from another point of view, as it shows that, at a very early stage, immediately after fertilization, if not before, the planes and regions of the potential embryo are embodied in the structure of the egg in definite relations to space. This property, which may, for convenience, be called molecular organization as distinguished from cellular organization, may be pictured as due to recent influences, or it may be imagined to be an inherent property of the ovum, inherited from the ovary of the parent.

Now all five eggs of Salpa hexagona lie, so far as their potential organization goes, in the same plane, for the plane of the fertilizing duct is also the plane in which each egg divides into a right and a left half. These eggs are greatly crowded at the time when they are shut into the body of the chain-salpa, as this is developed in the wall of the stolon, and as Plate XLV, Figs. 6 and 7, show, they are compressed and distorted into very irregular shapes, and the five eggs in the set cannot, at this time, maintain equivalent positions, nor can we suppose that they afterwards right themselves and assume similar positions under the action of gravity or some other influence which acts alike on all five, for they are so closely wrapped by the surrounding follicle that they are not free to move. So far as I can see, the only influence which affects all five eggs in the same way is the spermatozoon, which, as it passes up the lumen of the duct, must come into contact with the middle line of the proximal surface of each egg. The first plane of segmentation is the plane which passes through this point, and I think we are therefore justified in believing that the unfertilized egg of Salpa hexagona is not bilateral in inner structure, but that the plane of fertilization becomes the plane of symmetry. So far as I am aware, salpa is the only animal in which the definite plane of adult, bilateral symmetry can be proved to exist in the unfertilized egg, and I think that there is good reason for believing that it is not determined here by anything in the organization of the egg, but by a purely accidental condition; the fact that, as the opening of the fertilizing duct is a fixed point, fertilization can take place at only one spot. O. Schultz believes (Zur ersten Entwicklung des braunen Frosches) that the middle plane is fixed in the frog's egg before fertilization, but Roux (Beiträge zur Entwicklung-mechanic des Embryo, No. 4, Arch. f. Mik. Anat. XXIX, 1887, p. 207) holds that while the poles are determined by the distribution of the yolk, the egg of the frog is not differentiated with reference to any particular one of the meridians which pass through the poles, until after fertilization, when the plane of fertilization becomes

the plane of symmetry, as it does in salpa, although the point where fertilization takes place is not fixed as it is in salpa.

. Hatscheck holds, as is well known (Ueber Entwicklungsgeschichte von Teredo, Arb. Zool. Inst. Wien, 111, 1880, p. 25), that bilateral symmetry is present potentially in the molecular structure of the egg before fertilization, and that it is simply made manifest as development advances; but Metschnickoff (Vergleichend-embryologische Studien, 3, Zeit. f. Wiss. Zool. XXXVII, 1882, p. 303) has shown that this interpretation is unwarranted, and Davidoff (16, p. 581) agrees with Metschnickoff that the early appearance of bilateral symmetry in segmenting eggs must be attributed to secondary modification of the ontogenetic record.

Every naturalist knows that the bilateral animals have not inherited this characteristic from a common source, for there are abundant examples of the secondary acquisition of bilateral symmetry, as in the spatangoids for example, and in the pulmonate gasteropods, and in the bilateral species of anthomedusæ and in the siphonophores, to say nothing of the infusoria, and no one can believe that it is as old, phylogenetically, as fertilization or segmentation, and if this is true, as it certainly is, and if its early appearance is due to secondary acceleration, there is no reason why it should come into existence at the same stage in all animals. In some animals it may possibly be potential in the ovarian eggs and may even coincide with the symmetrical plane of the body of the parent, and if this should some day be proved we should still have every reason to regard it as secondary. In other animals it may remain indefinite long after fertilization, or as I believe, long after the gastrula stage. In salpa it is fixed by external limitations, but the observations of Seeliger (9), Davidoff (16), and Van Beneden and Julin, all show that the egg of clavelina does not manifest it until the four-celled stage is reached, and Metschnickoff says that in another ascidian, *Ascidia mentula*, no trace of it is exhibited until after the numerous cells of the blastula have become differentiated into large vegetative and small animal cells. I think that it is plain that if its early appearance is the result of secondary acceleration, we cannot deduce from the fact of its early appearance in the embryos of the bilateral, cœlomatous metazoa any conclusion whatever regarding their common ancestry.

CHAPTER XII.

THE ACCESSORY PARTS OF THE EMBRYO.

SECTION 1.—*The Composition of the Embryo.*

Besides the blastoderm cells and their derivatives, the germ layers, the salpa embryo contains a number of structures, which are partly nutritive and partly protective, and the greatest peculiarity of its development is the very complex way in which these various parts become involved with each other. Their inter-relations are of such a character that it is not easy to give a clear description of one of them without continual reference to the others, nor is any general description of the whole possible until the reader has gained some knowledge of the history of the various constituents.

The composition of the embryo has been described in outline in the chapter on the embryology of salpa, and I shall now describe each structure by itself, with such references to other parts as may be necessary. This plan will involve much repetition, but it seems on the whole to be the simplest and the least fatiguing to the reader, although I fear that the history cannot be set forth in such a way as to be perfectly clear at the first reading.

The accessory structures are of two sorts: those which are derived from the follicle = annéxés de l'embryon, Barrois (6), p. 467; and the fœtal membranes. I shall describe them separately—the fœtal membranes first.

SECTION 2.—*The Fœtal Membranes = Annéxés extra-fœtales, Barrois, (6), p. 467.*

I have nothing to add to the clear and simple account of the mode of origin of the fœtal membranes, and their anatomical relations, which have been given by Salensky (5) and Barrois; but my own observations on the *fate* of these structures are, in many respects, so different from the statements which are made by these authors, that it will be necessary

to preface my account of my new observations by a short outline of features which have already been so well described.

The unfertilized egg in its follicle, Plate X, Fig. 1, lies in the body cavity of the chain-salpa, where it is suspended from the wall of the atrium by the fertilizing duct, which shortens after fertilization in such a way as to draw the egg up to the point where the duct is attached to the wall of the atrium. The way in which the egg reaches its final position by the shortening of the suspensorium has been well described in minute detail by Leuckart (1), p. 48; by Salensky (5), pp. 183-5, and by Barrois (4).

While this change is taking place the embryo begins to grow and to project into the atrium, pushing its wall inwards as shown in Plate IX, where b, colored red, is the wall of the atrium, while the yellow tint marks the body cavity.

The embryo, in its follicle, colored blue, is then folded into an *epithelial capsule*, which at first consists, like the wall of the atrium and pharynx, of very flat cells, Plate X, Fig. 6, b', colored red in the figures. Soon the cells immediately around the point where the egg is fastened, Plate X, Fig. 9, *11*, become thickened and columnar, Plate X, Fig. 9, b', and thus become converted into the "Epithelhügel" of Salensky. The patch of thickened epithelium gradually spreads on all sides until it ultimately covers the whole embryo, Plate XI, Figs. 1, 2 and 3, b', except within the area which is shown in Fig. 3, between the 7 and the F of the word Fig. This space never becomes covered by the capsule, and it ultimately becomes the roof of the placenta, Plate XVIII, *10*. As regards the origin of the epithelial capsule, Salensky says (2), p. 182, that it is nothing more than the thickening of the wall of the pharynx, but this is certainly an error, although of little significance. The egg is not fastened to the wall of the pharynx, but to that of the atrium. As I did not reach conclusive proof of this error until after my drawing had been finished for photolithography, I have used the colors and reference letters for the atrium in these figures which are used elsewhere in this memoir for the pharynx.

It is possible that the epithelial capsule may be part of the wall of the atrium, but I have already given my reasons, page 236, for believing that the suspensorium, Plate X, Fig. 9, *11*, may, while shortening, possibly spread out at its mouth and give rise to the area of thickened epithelium, b'. If this be the case, the epithelial capsule is follicular in its ultimate origin.

Barrois says (4), p. 467, that he has not been able to decide whether it is the thick border of the opening of the oviduct or a new formation.

Plate XI, Fig. 3 shows the embryo in its follicle projecting into the atrium *c*, and inclosed in the epithelial capsule *b'*, which is continuous at the bottom of the figure with the epithelium of the walls of the atrium. At a stage a little older, Plate XLI, Fig. 1, *23*, it runs down for some distance beyond the outline of the embryo, and forms the wall of the placenta. The figures *24* and *31* are in the cavity of the *placenta*. At a still lower level the capsule is constricted to form the narrow *neck* of the placenta, through which its cavity communicates with the body cavity of the chain-salpa.

In Salpa hexagona, Plate XI, Figs. 4, 5, 6, 7, 8, *b'*, that part of the capsule which incloses the embryo is not in close contact with it, but separated from it by a space which is well shown in the figures.

In Salpa pinnata, on the other hand, Plates XII, XIII and XIV, *b'*, it is so closely applied to the follicle cells of the embryo, at least in specimens which have been hardened and imbedded for sections, that very careful examination of the horizontal sections is needed to find it.

SECTION 3.—*The Supporting Ring.*

As shown in Plate XLI, Fig. 1, the cells of that portion *23* of the epithelial capsule which lies below the level of the embryo soon become elongated, so that the wall here becomes much thickened to form that portion of the placenta which I shall call the supporting ring; the structure which is shown at *23* in the figures. It is well shown in the embryo of Salpa hexagona at *23* in Plate XLI, Figs. 2, 3 and 4, and in Salpa pinnata, in Plate XXII, Plate XVIII and Plate XXXV, as well as in the woodcuts on pages 29, 30 and 31.

At first the transition from the thinner portion *b'* which covers the embryo to the thickened portion which is to form the supporting ring *23*, is gradual, as is shown in Plate XLI, Fig. 1, and in the diagram in Plate XII, Fig. 11. The line between the two regions soon becomes abrupt and strongly marked, cut B, and in Salpa pinnata, XXII, Fig. 1, the upper edge of the supporting ring soon bends inwards towards the axis of the placenta in such a way as to interrupt its continuity with the portion around the embryo.

In Salpa hexagona this continuity persists, Plate XLV, Fig. 4, *23* and *B'*, as long as the epithelial capsule is retained, and the history of the

supporting ring is very simple in this species, where its function is to give support, like a pessary, to the embryo at its point of attachment to the wall of the atrium, and by its rigidity to maintain a free channel for the circulation of the blood through the narrow neck of the placenta into and out of its cavity. This appears to be its primary function in all the species, as Barrois points out in the case of Salpa africana. He says, p. 495, its chief function is to support the embryo and its membranes, upright in the middle of the uterine cavity (cavity of embryo sac). In this species the inner ends of its cells soon become dotted by a row of circular spots which are shown in Figs. 3 and 4. These spots are so much like the muscle fibers seen in sections of the tissues of cœlenterates, as to suggest the thought that they may form a sphincter of epithelio-muscular fibers around the neck of the placenta.

I have had no fresh specimens for teazing, and my series of embryos is so scanty that I have not had the material for further investigation of this point, although it seems of sufficient interest to deserve the attention of histologists.

Section 4.—*The Embryo Sac.*

It is well known that in most species of salpa the embryo becomes inclosed in a brood chamber or incubatory pouch which is formed about it by a circular collar-like fold in the wall of the cloaca, around the area where it is attached.

While the fold is not formed out of the tissues of the embryo, like the amnion of insects and the higher vertebrates, its mode of origin and its anatomical relations to the embryo are so similar that the word "amnion" comes naturally to the mind, although the term embryo sac seems better.

Around the neck of the placenta the epithelial capsule is at first continuous with the ordinary epithelium of the atrium, as shown in Plate XI, Fig. 1, but soon a circular collar-like fold, Plate XLV, Fig. 2, *21* and *22*, is formed around the area of attachment. This fold is the amnion, or according to Salensky terminology, the "Faltenhülle."

It never attains to any considerable size in Salpa hexagona, and Salensky states that it is absent in a very similar species, Salpa democratica. It is shown in three successive stages in Salpa hexagona, at *21* and *22*, in Plate XLV, Figs. 2, 3 and 4.

In Salpa pinnata, at a very early stage, the amniotic fold grows up on all sides of the embryo until this is completely shut in to a brood

chamber or embryo sac, Plate XVIII, Figs. 4 and 8, with only a minute opening into the atrium at the top.

The difference between Salpa hexagona and Salpa pinnata will be seen by comparing Plate XVIII, Fig. 4, which shows the brood sac in its most perfect condition, and Plate XLV, Fig. 4, which shows the greatest degree of development which it ever reaches in Salpa hexagona. The embryo sac may be rudimentary as it is in Salpa hexagona and Salpa democratica, or fully developed as it is in Salpa pinnata, but I think there can be no doubt that it is strictly homologous in all the species. Salensky believes, however, that its mode of origin is totally different in different species, and he says, page 152, that its relation to the other parts of the embryo of Salpa africana is very interesting, since it is a constituent part of the embryo and does not arise from the wall of the branchial sac, as it does in other species.

It is rather surprising, however, to find a few pages farther on, page 157, the statement that his own observations of this species completely confirm Barrois' account of its origin, for Barrois says that it is formed in Salpa africana (maxima) from the walls of the branchial sac, exactly as Salensky himself describes it in Salpa pinnata.

To recapitulate: the area around the point where the embryo is attached to the wall of the atrium becomes folded about the embryo in such a way as to form an epithelial capsule which covers the surface of the embryo; a supporting ring which forms the side walls of the placenta; and an embryo sac which, in Salpa pinnata and in many other species, completely covers the embryo. The brood chamber, or the space inside the embryo sac, is part of the atrial chamber of the chain-salpa, and in Salpa pinnata it opens into the chamber by a small duct; while the space between the two folds of the amnion is part of the body cavity of the chain-salpa. Comparison of cut B or Plate XVIII, Fig. 4, with the horizontal sections in Plates XII, XIII and XIV, will show that a horizontal section must pass: 1st, through the outer fold *21* of the embryo sac; 2d, through the space *y'*, between the two folds, part of the body cavity of the chain-salpa; 3d, through the inner fold of the embryo sac, *22*; 4th, through the brood chamber *25*, which is part of the dorsal chamber of the chain-salpa; 5th, through the epithelial capsule *b'*, and 6th, through the embryo in its follicle.

SECTION 5.— *The Fate of the Fœtal Membranes.*

I. *The embryo sac.* This undergoes little change after it is fully developed, and it does not grow with the growth of the embryo. It is formed in Salpa pinnata at a time when the embryo is very small, and growth causes the embryo to push up through its opening, which is thus greatly distended, into the atrium, as is shown in Plate XLI, Fig. 2, and in Plate XXXV, where the entire body of the salpa is outside the embryo sac, which reaches only to the level of the top of the placenta. In fully grown embryos the embryo sac is so small in comparison that it is scarcely discernible with any magnifying power which can be used. At birth the rupture which sets the embryo free takes place, in Salpa pinnata at least, between the lower edge of the supporting ring and the lower edge of the inner fold, which is thus left behind when the embryo with its placenta drops into the pharynx of the chain-salpa, to escape into the water.

II. *The epithelial capsule.* My observations on the fate of the epithelial capsule are totally different from Salensky's. He says (389) that in Salpa bicaudata and Salpa democratica it is transitory; while in Salpa africana, pinnata, fusiformis and punctata, it becomes converted into the ectoderm of the embryo. I have studied only one of the species in his list, Salpa pinnata, but I have studied Salpa hexagona, which is a representative of the democratica group, and I find that in these two species it is a temporary deciduous structure, as it is also in Salpa africana according to Barrois (6), p. 478, and in Salpa democratica and Salpa bicaudata, according to Salensky. In these species it has no share in the formation of the embryo. It covers it like an ectodermal epithelium, but as the true ectoderm is gradually developed beneath, it is pushed off or moulted.

In both these species the ectoderm is blastodermic in its origin, and it is formed, as I shall show later on, from certain ectodermal blastomeres, Plate XVII, Fig. 5, *9* and *9'*; Plate XXII, Figs. 3 and 5, *a''*; Plate XII, Fig. 10, *9'*, and cuts A and B, *A'*, which at a very early stage of development migrate out of the follicle and come to lie immediately under the epithelial capsule, *b'*.

The history of the epithelial capsule is as follows: At first, Plate XI, 1, 2, 3, *b'*, it is nearly uniform in thickness and made up of columnar cells, but as the embryo grows it is stretched so much along its sides that the cells here become very flat, Plate XXII, Figs. 1 and 2, *b'*, and Plates

XII, XIII and XIV, while it remains thick for a very much longer time near and upon the middle line of the dorsal surface of the embryo, as shown at the top of Figs. 5 and 6 of Plate XXII, and still better in the horizontal sections, Plate XVII, Fig. 5, *b'*, and Plates XIII and XIV, Figs. 1 and 2, *b'*. The true ectoderm first appears on the middle line underneath this thickened portion of the epithelial capsule, as shown at *A* in cut D, and very highly magnified, from another section of the same specimen in Plate XLII, Fig. 11, where *21* is the outer and *22* is the inner fold of the embryo sac, *b'* the cells of the epithelial capsule, and *a'* the ectoderm. As the ectoderm grows and spreads over the embryo it at first pushes under the cells of the epithelial capsule and crowds them out and stretches them apart as shown in the last figure, and in Fig. 9 of the same plate where *a'* is the ectoderm and *b'* the cells of the epithelial capsule. As the ectoderm becomes more developed many of these cells are often pushed along before its growing edge and crowded together in an irregular pile, as shown at *b'* in Plate XVII, Fig. 4 and Fig. 1. The details of the process of moulting vary greatly. In some specimens big scales and flakes of epithelium are dropped off and are found around the embryo, but as a rule the cells separate from each other before they become detached and they drop off separately. The moulted cells are shown from Salpa hexagona at *B'* in Plate XLV, Fig. 3, and in Salpa pinnata in Plate XVI, Figs. 2 and 3, at *b'*. Fig. 6 of this plate is a portion of the ectoderm from another section of the same embryo very much magnified; *21* and *22* are the outer and inner folds of the embryo sac; *25*, the brood chamber; *b'*, the cells of the epithelial capsule; *a*, the ectoderm, and *15*, the body cavity with amœboid follicle cells. When the cellulose mantle first makes its appearance it is a thin transparent layer, Plate XLV, Fig. 3, *v*, in contact with the ectoderm, and carrying on its outer surface the scattered cells, *b'*, of the epithelial capsule. These cells soon drop off, however, and at a stage a little older the outer surface of the cellulose mantle, Plate XLV, Fig. 4, *v*, is naked, although a cell, apparently from the epithelial capsule, may occasionally be found embedded in it, as shown at *v* in Plate XVII, Figs. 6 and 7.

III. *The supporting ring.* As regards the fate of that part of the epithelial capsule which I have called the supporting ring of the placenta, *23* of the figures, there is a very important difference between Salpa pinnata and those stages of Salpa hexagona which I have studied. In the latter species it is, up to the time of birth at least, simply a thickened ring-like support or pessary for holding the embryo and the

placenta in position, and for maintaining a free open channel for the blood which circulates into and out of the placenta. In Salpa pinnata it not only serves this purpose, but it is also a most remarkable mechanism for the nutrition of the embryo, although it effects this indirectly; furnishing by means of a most noteworthy and remarkable process of migration and degeneration, the food for the follicle cells of the roof of the placenta, which, in their turn, migrate into the body cavity of the embryo and there degenerate.

The nutrition of the salpa embryo is a most interesting field for research, and I have not been able to devote to it enough space nor a sufficient number of figures to give more than a general outline, but I hope that this will incite some one to more exhaustive study of the subject.

In Salpa pinnata, as I have stated, the supporting ring soon bends inwards towards the roof of the placenta, losing its continuity with the epithelial capsule, and becoming very intimately united to the roof of the placenta, as shown in Plate XXII, Fig. 1. Its cells, which are bathed by the blood which circulates in the placenta, grow and multiply with most remarkable rapidity by direct division. Cell multiplication goes on so fast that every nucleus in each section is in some stage of division, and no resting nuclei are to be found.

This is well shown at 23 in Plate XXXV, and more highly magnified in Plate XLIV, Fig. 3. Growth takes place with equal rapidity, and as the cells grow they move upwards towards the roof of the placenta, their nuclei, which keep on dividing, growing to an enormous size, and becoming so filled with chromatin granules that in stained specimens they are almost opaque. At the upper edge of the supporting ring the cells separate from each other and degenerate, giving rise to a stream of deeply stained granules, irregular particles, and fragments of nuclei, which are swept along, by some means which I have failed to discover, towards the central region of the roof of the placenta, where they serve as food for the enormous placenta cells which are shown at 29 in Plate XVIII, Fig. 5, and in many of the other figures. It seems probable that after the embryo of Salpa hexagona is born the cells of its supporting ring are used up as food in the same way, but I have seen no indication of this before birth, although the growth and migration of the follicle cells, Plate XLV, Figs. 2, 3 and 4, 29, are as conspicuous in this species as they are in Salpa pinnata.

SECTION 6.—*The Literature of the Fœtal Membranes.*

I have tried to tabulate the various technical terms which have been applied to the fœtal membranes and to the parts of the placenta by the more recent writers on salpa, and as a list of these synonyms is a necessary aid to the reader, I print it here, the more willingly as it will serve the purpose of a brief summary of the less important differences of opinion.

Epithelial capsule = cul-de-sac of Barrois (6). According to Barrois, the primary epithelial capsule, before it becomes divided into the epithelial capsule proper and the supporting ring, is the *primitive cul-de-sac*, and after the differentiation the epithelial capsule in the strict sense is the *cul-de-sac.*

= Inner lamella of brood chamber (in Salpa democratica), Salensky (7).

= schildförmigen Verdickung, Leuckart (9), p. 47.

= Epithelhügel and Epithelialhügel, *eph,* Salensky (12), for the undifferentiated structure.

= Ectodermkeim, Salensky (12), *eck,* for the epithelial capsule proper.

= Primitive uterus, and internal decidua of uterus, Todarro.

Supporting ring = Couche périphérique du placenta, Barrois of placenta   (6).

= Placenta, *Pl.,* Salensky (12), p. 100.

= Randwulst der Placenta, Salensky (12), p. 102.

= Direct decidua, Todarro.

= Placental membrane, Todarro.

= Cerchio blastodermico, Todarro, for its inner edge.

Neck of placenta = Peduncle, Barrois.

Brood chamber = Uterine cavity, Barrois (6), p. 425, and Todarro.

Embryo-sac = Decidua reflessa, Todarro.

= Faltenhülle, Salensky (12), *Flh* or *Fhl* or *Fh.*

= Amniotic membrane, Todarro, for inner fold.

= Reflected portion of the uterus, Barrois for outer fold.

= The two folds together make the decidua reflessa of Todarro.

= Uterus, Barrois and Todarro, for the cavity of the embryo sac.

= Its aperture is the neck of the uterus, Todarro.

= The space between the two folds, y', in my figures, is the amniotic cavity of Todarro.

So far as the origin and anatomical relations of the fœtal membranes are concerned, the account which I have given is little more than a repetition of the accounts which have already been published by Barrois (4) and Salensky (5), and their descriptions show that while the various species present slight differences of detail, especially as regards the degree of development which is reached by the embryo sac, the history is essentially the same in Salpa democratica, hexagona, africana, fusiformis, pectinata, and pinnata, or in all the species which have been studied, with the possible exception of Salpa scutigera (bicaudata).

Salensky has shown that in each species the egg and the embryo have certain distinctive specific characters which might be used for the diagnosis of species, and the illustration and description of these specific features form one of the most interesting sections of his memoir, but it is to be regretted that he did not bring all these specific characteristics together in one place, so that the rest of his paper might be devoted to an account of the common characteristics of the salpa embryo. There can be no doubt that the general effect of his paper is to convey to the reader an exaggerated notion of the difference between the embryos of the different species, and this effect is all the greater since the terms which he uses in his description of Salpa democratica are quite different from those by which he designates the same parts in other species.

Careful examination of his first paper (2) will show, however, that so far as the fœtal membranes are concerned, the only difference between Salpa democratica and all the other species (possibly with the exception of Salpa scutigera) is the lack of the embryo sac, or, at least, its very scanty development; but as the wall of the embryo sac in the other

species takes no part in the formation of the embryo, and is very transitory, this difference is only a superficial one, and it leads to no complications, nor does it in any way affect the comparison between the embryos of different species.

The brood chamber, Brutsack, of Salpa democratica in Salensky's first paper (2), is obviously the cavity of the follicle which is designated in my figures of Salpa hexagona, Plate X, by *5*. It is the cavity which he calls, in his second paper (5), in all the species except Salpa democratica, the cavity of the follicle, and designates by the letters *Fh*, as in his Taf. 10, Fig. 9, *pin* for example. It is true that its walls are formed, in great part at least, from the cells of the duct rather than from those of the egg capsule, but his figure of the embryo of Salpa pinnata (5), Taf. 10, Fig. 9, shows clearly that this is not a fundamental difference, for in the latter species his figures show that the cells of the egg capsule and those of the duct are parts of a continuous layer of follicular epithelium without any sharp line of separation. I must not be held to deny the existence of the difference between the two species in this particular, since I simply wish to point out that, while real and interesting, it has no deep significance and does not involve any fundamental difference between the two embryos.

The inner lamella of the brood chamber of Salpa democratica, *Brs, α*, is simply the somatic layer of the follicle, designated in my Plate X and in other plates by *7*. It is the layer which, in his second paper, he has designated in other species by the letters *Fe*.

The outer lamella of the brood chamber, *Brs, β*, is, as he points out on page 378 of his second paper, the structure which I have called the epithelial capsule, *b'*, and that which he calls, in his second paper, the Epithelialhügel, *Eph*. The part of the follicle which in his first paper he designates by the letter *p*, and which, as he shows, forms the greater part of the placenta, is exactly the same as that part of the somatic layer of the follicle which in other species forms the roof of the placenta and is designated in this memoir by *10*. His figures of the embryo of Salpa democratica in his second paper, especially Taf. 27, Fig. 3, *dl*, show that the sides of the placenta are strengthened by a supporting ring, *Eph*, derived from the lower part of the epithelial capsule exactly as in the species which I have studied.

In his second paper (5), p. 378, he himself points out these facts, and his retention of one terminology for Salpa democratica, while another is used for the other species, unfortunately helps to impress the reader with an exaggerated notion of the difference between the species.

In his second paper he says that the Faltenhülle, or, as I have called it, the embryo sac, is lacking in Salpa democratica; but the figures in his first paper, especially Taf. XIV, Fig. 18, seem to indicate that this structure is actually represented by a slight fold, as I have shown it to be in Salpa hexagona, Plate XLV, Fig. 3. Whether it be totally absent or very fully developed, no complication is thus introduced, for even in the species where it is most developed, like Salpa pinnata and Salpa fusiformis, it takes no part in the formation of the embryo, and its presence or absence is a matter of minor moment. We may therefore state with confidence that, so far as the fœtal membranes are concerned, the various species of salpa (with the possible exception of Salpa scutigera, which I have not studied) are fundamentally alike, although each species has its own distinctive peculiarities, which often attain to such prominence as to be noteworthy.

Todarro's account of the fœtal membranes is very different from Salensky's or Barrois'; but, as Salensky has clearly shown, he has been misled by a purely fanciful resemblance between the salpa embryo and that of a mammal. His description has been so fully discussed by Salensky that nothing more on the subject seems to be called for.

As regards the fate of the embryo sac, my paper is simply a verification of the studies of Barrois and Salensky, but Barrois says little about the fate of the supporting ring and epithelial capsule, and while Salensky's history of the epithelial capsule (Ectodermkeim of salpa in general; outer lamella of brood chamber in Salpa democratica) is very explicit, it disagrees totally with my observations.

Barrois finds (4), page 478, as I do, that the epithelial capsule is thrown off, but his account of the minute details of the process in Salpa africana is somewhat different from my observations on Salpa pinnata and Salpa hexagona. He says, "The complete closure of the uterus (embryo sac) is the signal for a great and important change, which consists in the destruction of the cavity which has up to this time played the part of a cavity of incubation, the cavity of the follicle." "The membrane s (the epithelial capsule) which has up to this time been united to the area where the placental membrane (supporting ring) and the lower part of the wall of the follicle (roof of the placenta) are joined together, now loses its connection at this point and is thrown off, leaving the tissues of the embryo free in the interior of the uterus" (embryo sac).

He describes and figures it as thrown off in a large sheet, but this is certainly not the case in the species which I have studied. Some of my

sections show detached scales of considerable size, but more generally the cells separate from each other and are thrown off singly. Barrois says that he does not know what becomes of the epithélial capsule after it is detached, although he thinks that it may possibly become converted into the cuticle of the embryo.

Salensky believes that this fœtal structure which, in its origin, is not part of the embryo but part of the body of the chain-salpa, becomes converted into the ectoderm of the embryo, which is therefore derived, not from the egg, but from the wall of the atrium, or, as he states, that of the pharynx.

His account is so irreconcilable with my own that I shall quote it at length.

He says (5), p. 135, that, in Salpa pinnata, "The integument is developed out of the Ectodermkeim, which must therefore be regarded as a structure analogous to the ectoderm of other animals. We have seen that in the preceding stages the Ectodermkeim consisted, in the greater part, of a single layer of small flattened cells which become thickened and cubical at the top of the embryo. At a later stage the thickened area of ectoderm cells grows farther and farther down, until, over the whole surface of the embryo, the ectoderm cells have regained their original, cubical form. The cause of this change in the form of the ectoderm cells is, in my opinion, to be found partly in the difference in the rate of growth of the internal parts of the embryo as compared with the ectodermal layer, and partly in the production of the cellulose mantle. At the time when the ectoderm cells become flattened, we find that the mesoderm cells (cells of the somatic layer of the follicle) are multiplying, and thus greatly increasing the size of this layer. After the proliferation of the mesoderm cells has come to an end, and the secondary cavity of the follicle has become filled up with these cells, the growth of the ectoderm begins; and this stands in obvious relation to the formation of the cellulose mantle."

"This latter is formed very early in Salpa pinnata, and its first stage is the division of the cells of the Ectodermkeim, which precedes the growth of the ectoderm cells. I need hardly note that the cellulose mantle is formed from ectoderm cells, as Hertwig, Arsenieff, Todarro and the author have shown. In Salpa pinnata we find before the formation of the cellulose layer, the division of the ectoderm cells which separate later from the ectoderm and pass into the clear homogeneous cellulose. This layer is first formed at the upper end of the embryo

where the ectoderm cells are the longest, and it afterwards extends downwards. Since this process involves the multiplication of the ectoderm cells, and since this multiplication and division follows growth, we can understand that the construction of the cellulose mantle which begins on the upper surface of the embryo must, as it extends downwards, bring about the growth of the ectoderm cells in the lower part of the embryo."

It will be seen that Salensky's view is that the epithelial capsule (Ectodermkeim) is at first thick and made of cubical cells; that it becomes stretched and its cells flattened on the sides of the body by the distention which is produced by the growth of the internal parts, and that it afterwards becomes thick again to form the true ectoderm.

My own observations show that at first it is uniformly thick; that it becomes distended at the sides by the growth of the embryo, and that its cells are then thrown off as the true ectoderm becomes developed under them.

Salensky's exhaustive researches on so many species are worthy most careful consideration, and I have not approached the subject with any preconception, for I am fully prepared to believe, on sufficient evidence, that the ectoderm of the salpa embryo comes from the tissues of the body of the chain-salpa rather than from the egg.

I think, however, that my observations furnish two reasons for believing that the epithelial capsule is thrown off as I have stated, and that it takes no part in the formation of the embryo. In the first place it will be seen that the flattened cells which cover the sides of the embryo in the stage shown in Plate XXII, are thrown off in the flattened condition, as shown in Plate XVI, Fig. 6, *b'*, and that they never become cubical, but are different, even after detachment, from the true ectoderm cells. In the second place it will be noticed that after the cellulose layer, Plate XLV, Fig. 3, *V*, has made its appearance, these cells are often to be seen scattered over its outer surface, as shown at *B'*, and not in its substance.

CHAPTER XIII.

THE FOLLICLE AND THE PLACENTA.

While Todarro seems to have observed the proliferation of the follicle cells of salpa, Salensky is the discoverer of their most remarkable and complicated relation to the developing embryo.

His memoir (5) is the first great step towards a true insight into the embryology of salpa, and it must always hold a most prominent place in the literature of the subject.

My own work has been rendered much more simple by Salensky's account, which has aided me so much that I cannot but regret the hostile attitude which I have been forced to assume towards the most characteristic opinions of its author, although I believe that a critical review of his own account of the facts will lead the reader to adopt my interpretation of them.

SECTION 1.—*The Origin of the Follicle.*

At the time of fertilization the egg is inclosed in an epithelial capsule of follicle cells, the history of which, as already described, pp. 214 and 225, is as follows :

The germinal mass which is formed in the body of the young solitary salpa, at the place where the proliferous stolon is afterwards developed, is, at first, a compact mass of undifferentiated cells, as shown in Plate XX, Fig. 6, at *n*, and more magnified in Plate XLI, Fig. 7. As the stolon grows, this mass becomes elongated into a rod, as shown in Plate XVI, Fig. 5.

At first, cells in all parts of the germinal mass show signs of multiplication, but the peripheral ones soon become differentiated into an epithelial layer, Plate XX, Fig. 2, *m*, Plate XXI, *m*, and Plate XXXI, Figs. 4, 5, 6, 7, 8 and 9, and a central core of germ cells, which latter lose the power of multiplication which is retained by the peripheral cells, and become converted into eggs. As the bodies of the chain-salpæ become marked off in the walls of the stolon, the epithelial layer also grows inwards, as shown in Plate XV, Fig. 2, *m*, Plate XXIII, Fig. 5, *m*,

Plate XXV, Fig. 6, *H*, *m*, and Plate XXXII, Figs. 7 *M* and 8 *N*, while an outgrowth from the follicle in the middle line of the body of the chain-salpa gives rise to the fertilizing duct, Plate XXV, Fig. 7, *H*, *m*, by means of which the egg becomes attached to the wall of the atrium of the chain-salpa, as shown in Plate XXXIX, Figs. 2, 3, 4 and 5 at *x*.

Salensky's observations show that, before the development of the embryo begins, a part at least of this duct becomes merged with the follicle, so that the capsule of the fertilized egg consists of the follicle plus part of the duct of younger stages.

SECTION 2.—*The Nutrition of the Eggs.*

The follicle is bathed directly by the blood which circulates in the ventral blood space of the stolon and in the bodies of the chain-salpæ, and, at a very early stage, some of its cells become differentiated from the others, as shown in Plate XXXI, Fig. 7. Their nuclei become enlarged, and the chromatin loses its definite form and becomes vaguely diffused, so that the nucleus now takes an uniform color from staining fluids. These modified cells I regard as yolk cells, which, having become charged with food absorbed from the blood, and having lost their vegetative power, wander in among the egg cells, where they become irregular and amœboid, as shown in Plate XXXI, Fig. 5, and soon degenerate and break up into irregular, deeply stained globules and granules, Plate XXXI, Fig. 6, which are assimilated by the growing egg cells, and again laid down in their protoplasm as yolk granules.

These migratory follicle cells are most abundant near the root of the genital string, where the young eggs are growing rapidly, but they become less numerous as the eggs grow larger, and they are seldom found in the budding section of the stolon.

No one could examine sections of the young stolon of any of the species which I have studied without finding them, although they have received little notice. They are quite different from the abortive eggs which occur in older stolons of Salpa democratica and in its young buds.

The migration and degeneration of follicle cells around a growing egg as a provision for its rapid nutrition is not at all unusual nor remarkable, and the evidence that it takes place in salpa is clear and conclusive. It would not be necessary to dwell upon or to defend this simple and natural interpretation of the facts, were not every part of the life-history of tunicates a source of dispute and of contradictory and

irreconcilable differences of opinion. I shall not enter the interminable and unprofitable "test cell" controversy, for however the case may be in other tunicates, the deeply stained wandering amœboid bodies which are found among the young eggs of salpa are certainly migratory follicle cells.

There is, however, another more important subject of controversy which we must soon take up—the fate of those follicle cells, or, to use Salensky's term, *Kalymmocytes*, which, at a later stage, migrate into the substance of the growing embryo of salpa.

It is well known that this observer has been led by his minute researches on salpa and pyrosoma to the view that the kalymmocytes contribute to the cellular structure of the embryo, so that the tissues of its body are, in part at least, derived from the follicle and not from the fertilized egg.

In this connection all new facts regarding the fate of any of the migratory follicle cells of salpa become important, and for this reason I shall quote a few observations from among the many which are on record, to show that my view of the way the egg of salpa is nourished is in accord with our knowledge of the history of the egg in many other animals.

Seeliger says (Eibildung und Knospung von Clavelina lepadiformis, Sitzb. der k. Akad. der Wissensch. LXXXV, I, 1882, p. 6) that only one cell in a certain area of the ovary of clavelina becomes an egg, while the cells around it aid in its progress towards completeness and lose themselves in it; and his paper is fully illustrated with figures to show the migration of the cells around the egg into its substance, and their degeneration to supply the material for the yolk.

Beddard says (Quart. Journ. Mic. Sc. XXX, p. 471) that in the majority of the oligochæta the ovum is detached from the ovary and falls into the egg sac in company with a number of germinal cells, which probably serve for the nutrition of the ovum.

Shelden says (Quart. Journ. Mic. Sc. XXX) that in Peripatus Novæ-Zealandica there is a small amount of yolk present in the young ovarian eggs, in the form of scattered spheres throughout the protoplasm of the ovum, and that there are also in the protoplasm small, round or oval nuclei, which in every respect resemble those of the follicle, so that it seems almost certain that these must have migrated from the latter, a process which would be simple in the absence of any separation between the ovum and the follicle. Hamann says (Anatomie der Ophiuren und

Crinoiden, Jenaische Zeitschr. XXXII, p. 278) that in the ophiurans the egg cell is surrounded by flattened follicle cells, which are, in origin, germ cells which have not developed into eggs. They are often found breaking down, and their cell substance thus affords the nutritive material for the growing eggs.

It is not necessary to give more quotations, for these are enough to show that there is abundant analogy for my interpretation.

The Migration of the Follicle.

The nutritive function of the follicle cells, which is established at this early stage in the history of the salpa egg, becomes very important after the egg is fertilized; and the migration of the follicle cells into the embryo, which is now to be described, is certainly one of the most remarkable phenomena in the life-history of salpa. Todarro seems to have observed it, but its importance as a dominant factor in the embryology of salpa was first recognized by Salensky, who has made a minute study of the subject, and has discussed it, at great length, in his memoir on the embryology of salpa (5), and more recently in his paper on the embryology of pyrosoma (17). The discovery of the remarkable history of the follicle cells is one of the most important steps towards a thorough comprehension of the life-history of salpa, and, in itself, it is enough to give to Salensky's memoir a most prominent place in the literature of the subject, although my own observations, while confirming his account of their origin, have forced me to differ from him regarding a most essential and fundamental feature, the fate of these cells.

I regard the cells which migrate into the embryo as exclusively nutritive, like those which migrate into the egg, while it is well known that Salensky holds that they become incorporated in the tissues of the embryo.

At the end of this section I shall state and discuss his views, but at present I shall confine myself to the presentation of my own observations.

In a ripe unfertilized egg, Plate X, Fig. 1, the follicle, which is colored blue in the figures, is one cell thick, and over most of the surface of the egg the cells are nearly cubical, although they become larger as they approach the region of the duct, x. Their nuclei are subspherical, with a fine network of chromatin. Here and there, in the sections, a cell is found in process of division, but cell multiplication is

not very active. When it does occur at this time it takes place by karyokinesis. At this stage the egg fits closely into the cavity of the follicle and fills it completely, but immediately after fertilization it becomes distended or enlarged, as shown in Fig. 3, so that the egg fills only about half of it, and there is an empty space, the cavity of the follicle, 5, which, in Salpa hexagona at least, is distal or opposite the pole where fertilization takes place.

The cells of the wall of this cavity now begin to multiply rapidly by karyokinesis, and nuclear figures may be found in every section, although they are at first confined to that hemisphere which is empty, while that which holds the egg retains for some time longer its epithelial character. The sharp outlines between the cells disappear in the proliferating portion, and the nuclei, which have become enlarged and are about to divide, are pushed inwards towards the inner surface of the follicle, as shown in Plate X, Fig. 3. In this figure the follicle is divisible into two sharply contrasted portions, 10 and 7. The hemisphere on the right, 10, is one cell thick and the outlines are distinct. The other hemisphere, 7, which for reasons which will appear later I shall call the somatic layer of the follicle, quickly becomes very much thickened by the elongation of the cells and the rearrangement of the nuclei, which no longer lie at uniform distances from the outer surface. The thickening is the result of crowding due to cell multiplication, but it does not result in the production of more than one stratum of cells. The proliferation of follicle cells is most active around the equatorial belt where the two hemispheres meet at the point marked 8 in Fig. 3 and in the following figures. At this point the cells soon begin to push inwards, as shown in Figs. 5 and 8, and to cover up the blastomeres 9, which have in the meantime been formed by the segmentation of the egg. These are soon completely covered and separated from the cavity of the follicle, Plate X, Fig. 8, 5, by a visceral layer of follicle cells, Fig. 8, 8. This layer appears to be formed from cells which migrate in from the wall of the follicle, although I found in one specimen, Plate IX, Fig. 11, 6, a clearly marked invagination of the wall. I do not know whether this is constant or not. I found no trace of it in other specimens, and Salensky, who has studied the follicle with great minuteness, says nothing of an invagination. It may not be a normal occurrence, although it is so definite and so well marked in this single specimen, that the failure to discover it in others may possibly be due to a failure to obtain sections of a proper stage in the right plane for showing it.

The cells of the visceral layer not only cover up the blastomeres, but soon push in between them and separate them from each other, as shown in Plate X, Fig. 8, in such a way as to form a solid mass of cells, which we may well call, with Salensky, the embryonic mass. It is made up, as Salensky has shown, of blastomeres and migratory follicle cells in the most intimate relation to each other.

We cannot follow by sections the path which each migratory cell takes, but it seems probable to me that those follicle cells which push in between the blastomeres come from the area marked *10* in Plate X, Fig. 8, where the somatic and splanchnic layers of the follicle are continuous with each other, while the cells which cover up the blastomeres push in around the zone which is marked *8* in Figs. 3 and 5, Plate X. The persistency of the cell boundaries in the area *10* may be due to the fact that the plane in which they move during migration is perpendicular to the surface of the follicle, while the proliferating cells of the somatic layer of the follicle, *7*, must move along parallel to the surface for a considerable distance before they reach the zone where migration takes place.

The embryonic mass grows by the addition of new follicle cells until it completely fills up and obliterates the follicular cavity, as is shown in Plate X, Fig. 9, and Plate XI, Figs. 1 and 2, where the somatic layer, *7*, of the follicle and the splanchnic layer are in contact with each other. While the change is taking place the migration of cells from the somatic layer gradually comes to an end everywhere except within the area *10*, where the two layers are continuous with each other. The somatic cells gradually become polygonal, their walls clearly defined, and they thus come to resemble those which make up the whole follicle before fertilization, as will be seen from a comparison of Plate X, Fig. 1, with Fig. 9. In this last figure the area, *10*, where the two layers are continuous, is the only place where migration still goes on.

It is important to note that the cubical, sharply defined, somatic follicle cells of the later stage are separated from the cubical cells of the follicle before fertilization, by an intervening period, when *all* follicle cells show traces of active change and a share in the process of proliferation and migration.

The study of the later stages shows that, during this period of active change, *the whole follicle, which was at first outside the egg, has become internal to the embryo, and is now, morphologically, in its body cavity.*

I have given, in the section on the morphological significance of the

salpa embryo, my reasons for believing that the follicular structures, which are colored blue in Plate X, Fig. 8, form a model or cast of the inside of a gastrula, although, as the result of unexampled secondary changes, the formation of the germ layers of the gastrula has been so much retarded that it has not yet begun.

It is important to note also that while all the follicle cells at the stages shown in Plate X, Figs. 5 and 8, exhibit every sign of an active share in the remarkable process of proliferation and migration, the actual movement, from without inwards, is confined to a restricted area, and it is also noteworthy that this area is considerably larger at first than at a later stage. At the stage of Plate X, Fig. 3, the area where migration is subsequently to take place includes about half of the spherical follicle or all of the hemisphere on the right of 8, 8, while at the stage of Fig. 8 migration is restricted to one pole, the one on the left in the figure. All these facts have been referred to in the discussion of the salpa embryo, and I have tried to show that they all help us to understand its true significance.

The subsequent history of the embryo shows that the area which is marked 10 in Plate X, Figs. 2, 5, 6, 8 and 9, is at the posterior end of its body near the middle line of its dorsal surface; that is, its relation to the body is the same as that of the blastopore of the chordata with an invaginate gastrula stage.

We have traced the history of the follicle to the stage when its cavity is obliterated, as shown in Plate XI, Fig. 2. The visceral portion, with its contained blastomeres, now fills it and lies in direct contact with the inner surface of the somatic layer, and the latter has become a thin, sharply marked epithelium, consisting of a single layer of cubical cells with distinct boundaries.

Part of another section from the embryo shown in Plate XI, Fig. 1, is shown very highly magnified in Plate XLII, Fig. 1. The somatic layer is on the left, where its nuclei form a single row. The outer ends of its cells form a continuous surface, while their inner ends are slightly rounded. The borders between the cells are distinct. The vigorous proliferation which took place at earlier stages has nearly ceased in the somatic layer, and when a cell is occasionally found in process of division, as at the top of the figure, this takes place by karyokinesis. The cells of the visceral layer, on the other hand, have no distinct borders, and they now begin to multiply very rapidly by direct division of their nuclei, which soon assume a most characteristic structure. They elongate and

become irregularly pear-shaped, the broad end being, in nearly every case, directed outwards, and the pointed end inwards. In most of the elongated nuclei the chromatic substance is gathered at the inner end in a mass which takes on a deep, diffused color from hæmatoxylin, while the rest of the nucleus contains only a few scattered granules. Cell multiplication, by direct division of the nucleus, now goes on so rapidly in the visceral follicle cells that each section shows many nuclei in process of division.

The somatic layer soon becomes distended and separated from the embryonic mass by a space which is shown, colored purple, at *15* in Plate XI, Fig. 3, and also at *15* in cut A, page 29, which is a reconstruction from a series of sections of Salpa hexagona at about the same stage as Plate XI, Fig. 3, but at right angles to that figure. This space is the body cavity of the salpa embryo, and it is marked *15* and colored purple in the figures of the later stages. It is bounded on all sides at this stage by follicle cells.

The embryo now becomes complicated by several important changes which will be described later on, although they must be briefly mentioned here to make the later history of the follicle intelligible. The first of the changes is the migration of the ectodermal blastomeres out of the follicle into the position where they are shown at *A″* in cut B. They become completely extra-follicular, and are covered only by the epithelial capsule *B′*. They are shown in Salpa hexagona in Plate XI, Figs. 4, 5 and 7, *9′*, and in Salpa pinnata, at *9′* in Plate XII, Figs. 1 and 2; Plate XII, Figs. 2, 4, 5, *a″*, and in other figures. In their migration to their final position they pass through the part of the follicle where the two layers are continuous with each other; the part which is marked *10* in Plate X.

The second complication is caused by the formation of the perithoracic tubes, which arise as a pair of invaginations of the somatic layer, shown at a very early stage in cut B at *G″*.

This cut was made from a series of actual sections, but they were cut in such an oblique plane that it seemed better to combine them in a diagram, than to draw a number of figures to illustrate a simple point. No single section cut both of the tubes, but the relations were as shown in the diagram.

The perithoracic tubes push their way in across the body cavity into the mass of visceral follicle cells, as is shown in cut B. This cut is not a diagram, but a careful reconstruction in a vertical plane from the series of horizontal sections of a young embryo of Salpa pinnata, shown in Plate XVII, Fig. 5, and in the figures in Plate XII.

The inner end of each perithoracic tube divides into two branches, a horizontal branch which meets and unites with its fellow, on the middle line, to form the atrium, G''' of the cut, and g''' of Plate XI, Figs. 4 and 5, and of Plate XII, Fig. 2, and a vertical branch, G of the cut, g' of Plate XII, Fig. 4, and g of Fig. 5, which ultimately opens as a gill-slit into the pharynx, as shown at G in cut C, and at c in Plate XIV, Fig. 9. After the atrium is formed by the union of the horizontal branches of the perithoracic tubes, these latter lose their external openings and their connection with the somatic layer; and they move inwards until they lie side by side on the middle line of the body above the cloaca, as shown in G'' of cut C, and also in Plate XIII, Fig. 6, g'', and in Plate XIV, Fig. 3, g''. It will be seen that as the result of these changes a complicated structure composed of follicle cells from the somatic layer is folded into the substance of the visceral mass of follicle cells.

The various organs of the body of the embryo now become mapped out in the follicle cells, as is shown by the series of horizontal sections in Plates XII, XIII and XIV; but as a knowledge of the details of this process is not needed to understand the later history of the follicle, they will not be noticed now.

The follicle reaches its greatest completeness at the stage which is shown in Plate XII and in cut B, and it will be necessary to examine these figures a little more minutely before we consider the fate of the follicle. The sections are from an embryo of Salpa pinnata a little younger than Fig. 1 of Plate XLI. Cut B, p. 29, is a careful reconstruction from all the sections. Comparison with the cut will show that the first section figured, Plate XVII, Fig. 5, cuts first on the right side the outer fold, 21, of the embryo sac, next the blood space, y, between the outer fold and the inner one, 22, which is separated in fresh specimens from the more central structures by the cavity of the embryo sac, which I have represented in the diagram, although the delicate unsupported folds are shrunken in specimens which have been imbedded in paraffine. Next comes the epithelial capsule B' or b', which is colored red in the figures. It will be noted that in this section the somatic layer of the follicle and the visceral layer are in contact with each other along the middle line, on what later stages show to be the dorsal surface of the embryo. The section next cuts the right half of the follicle above the level of the body cavity; next the ectodermal blastomeres, A', then the left half of the follicle and the top of the body cavity, and so on. The second section which is figured, Plate XII, Fig. 1 and cut B, passes

through the two folds of the embryo sac, *21* and *22*, and the epithelial capsule, *b'*, as before, and then on the right side it passes through the external opening of the right perithoracic tube. This opening does not communicate with the cavity of the embryo sac, however, since it is covered by the epithelial capsule. The section then passes through the follicular roof of the atrium, and after crossing the middle line it cuts the left perithoracic tube below the level of its opening, and after passing through the somatic layer of the follicle it cuts the epithelial capsule on the left side at the place where this is becoming thickened to form the supporting ring, *23*, of the placenta. This section shows that the somatic and visceral layers of the follicle are, at this level, in contact with each other at each end of the median axis, and later stages show that the bottom of this section is anterior and its top posterior. The section cuts the body cavity four times, twice on each side in front of and behind the perithoracic tubes. The two anterior divisions of the body cavity communicate at this level, but the two posterior ones do not, as the somatic follicle cells and the visceral ones are united at the posterior end of the middle line, as they are also around the openings of the perithoracic tubes.

The next section, Fig. 2, passes through the atrium, *g'''*, and the body cavity, *15*, which is bounded within by the visceral layer of follicle cells, *8*, and externally by the somatic layer, *7*. On the left side this section also cuts the top of the cavity of the placenta, *y''*. In this and the two following sections, *4* and *5*, the somatic follicle cells which line the atrium, *g'''*, and the perithoracic tubes, *g'*, are colored orange, like the blastomeres, as I did not discover their follicular origin until the plate had been made.

Figure 4 cuts the cavity of the placenta, *y''*, and the supporting ring, *23*, and it also cuts the two perithoracic tubes, *g'*, below the level of the atrium. At the level of this section the embryonic mass is almost completely separated from the somatic layer, *7*, and it is almost free in the body cavity, *15*. The next section, Fig. 5, cuts the right perithoracic tube near the bottom of its lumen, *g*, and the left below the lumen. The embryonic mass, which is free in the body cavity at this level, is divided into six well-marked regions, two of them paired and four on the middle line. The two paired structures are the perithoracic tubes, and each consists of a thick outer wall of visceral follicle cells and an internal epithelial of somatic follicle cells, colored orange in the figure. On the middle line there is an elongated mass or plate of visceral follicle cells, *8*,

with included blastomeres (*9*).   (The reference line which runs from the
(*9*) to the epithelium of the left gill tube is, as I have explained, the result
of an erroneous interpretation.)   Comparison of Fig. 5 with the sections
at higher levels will also show that this mass of cells hangs suspended in
the body cavity from the middle line of the dorsal surface, and that it is
pierced from side to side at the level of Fig. 2 by the atrium, *g'''*.   It is
divided by constrictions into four well-marked regions, and the later
history of the embryo shows that the lower one in the figure (*s*) is the
representative of the ganglion, and the second, which lies between the
gill tubes, that of the pharynx.   I shall give, further on, my reasons for
regarding the third (*19*) in Fig. 4 as the representative of the notochord,
and the uppermost (*18*) in Fig. 4 as the representative of the nerve of the
tail.   Comparison of Fig. 5 with Figs. 4, 2, 1 and Plate XVII, Fig. 5, will
also show that the blastomeres of the lowermost enlargement, *s*, of Fig. 5
and those of the uppermost, can be traced upwards until they become
continuous with the two ends of the string of ectodermal blastomeres
which lie outside the follicle on the middle line of the upper surface
covered only by the epithelial capsule.   In the next section, Fig. 6, the
cavity of the placenta, *y''*, almost completely surrounds the embryo and
its follicle, and the somatic follicle cells, *7*, are in contact with the
supporting ring, *23*, only at the two ends of the middle line.   It will also
be noted that the blastomeres of the region of the pharynx extend out
into the planes which are occupied at a higher level by the perithoracic
tubes, Fig. 5, *g*.

In the next section, Fig. 7, the cavity of the placenta, *y''*, which is
colored yellow, almost, and in Fig. 8 quite surrounds the embryo, and it
will also be seen that the somatic and visceral layers of the follicle are
united on the middle line at the anterior end of the body.   Sections 7, 8
and 9 are so simple that they will be intelligible without further explana-
tion when compared with cut B on p. 29.

The most noteworthy features of the embryo at this stage are these :
1. The blastomeres show no trace of an arrangement in germ layers,
although they are divided into an extra-follicular ectodermal set, *9'*, and
an endodermal set, *9*, imbedded in the visceral follicle cells, and form-
ing, with them, the embryonic mass.   2. The structure of the embryo is
vaguely outlined, but the outline is exhibited by the visceral follicle cells,
not by the blastomeres.   3. The cloaca and gill-tubes are more definitely
marked out, but they are entirely follicular, and consist of a thick outer
wall of visceral cells and an epithelial lining, derived from two paired

invaginations of the somatic layer of the follicle. 4. The dorsal hemisphere of the somatic layer of the follicle is closely wrapped by the epithelial capsule, cut B, except on the dorsal middle line where the ectodermal blastomeres lie between, and an approximately equal area of the somatic layer on the ventral hemisphere forms the inner boundary or roof, *10*, of the cavity of the placenta, *y'*, which separates this portion of the follicle from the supporting ring, *23*. 5. "A so-called blood bud" has been formed in Salpa pinnata at *24* in the cut, and in Fig. 9, as an outgrowth from the center of the ventral hemisphere of the somatic follicle. This brief description of the embryo of Salpa pinnata at this stage of development gives most of the facts which we need as a preparation for studying the history and fate of the follicle, but there are a few other embryological features which must be noted.

The two perithoracic tubes soon lose their external openings and their connection with the somatic layer of the follicle, and, as already pointed out, they move inwards until they come to lie side by side on the middle line above the atrium, as is shown in cut C and also in Plate XIII, Fig. 6, and Plate XXII, Fig. 4. Finally they degenerate, and the atrial aperture is formed as a new opening on the dorsal middle line, as shown in Plate XVII, Figs. 6 and 7. The cavity of the pharynx is hollowed out by the disintegration of the follicle cells of the visceral layer, as is shown at Plate XLII, Fig. 8, and the gill-slit or communication between the cavity of the pharynx and the cavity of the perithoracic tube is formed in the same way, as is shown at *x* in Plate XLII, Fig. 6, by the disintegration of the intervening layer of the follicle cells. The ectoderm, Plate XLII, Fig. 11, *a*, is formed by the growth and multiplication of the ectodermal blastomeres. It gradually stretches outwards and downwards, as shown at *a* in Plate XVIII, Figs. 4, 5 and 6, but it never covers up the ventral hemisphere of the somatic follicular layer. Even at birth the ectoderm is absent in the region of the placenta, and it is not until all traces of the follicle have disappeared after birth that the ectoderm covers up the spot which was occupied by the opening of the placenta.

SECTION 3.—*The Fate of the Follicle.*

The follicle may be divided, for purposes of description, into five regions, each of which has its own peculiar relations to other structures and its own peculiar history.

I shall show that, in every one of the regions, the follicle ultimately becomes disintegrated, loses its identity as a distinct tissue, and breaks up into wandering, amœboid follicle cells, which make their way into all parts of the embryo and gradually disappear.

While it is, of course, impossible to trace in sections the individual history of every one of these wandering cells, there is no evidence that any of them become converted into any of the cells of the embryo, while there is ample evidence that they are all used up as food, and that the function of all parts of the follicle is nutritive.

It will be best to treat each region of the follicle separately, and I shall speak first of the dorsal or embryonic portion of the somatic layer; second, of the somatic lining of the atrium and gill tubes; third, of the portion of the visceral layer which penetrates between the blastomeres; fourth, of the portion of the visceral layer which invests the blastomeres; and fifth, of the placental portion of the somatic layer of the follicle.

SECTION 4.—*The Disintegration of the Embryonic Portion of the Somatic Layer of the Follicle.*

I have shown, page 43, that the embryonic part of the somatic layer of the follicle separates at an early stage, from the part which forms the roof of the placenta, and that it soon afterwards breaks up into disconnected follicle cells, which separate from each other, and, becoming amœboid, wander into all parts of the body cavity of the embryo.

This is the region where the history of the follicle is simplest and easiest to follow, and where the evidence of its nutritive function is most obvious, and both Barrois' and Salensky's observations are consistent with my statement regarding it. Barrois, 473, says that this region of the embryo of Salpa africana consists of two layers; those which I have called the epithelial capsule and the somatic layer of the follicle; and that the latter soon disappears, although he was not able to satisfy himself whether it is actually destroyed or is simply fused with the inner layer.

Salensky has observed the phenomenon in Salpa pinnata and Salpa fusiformis (5), p. 130 and p. 352, and he has described it in words which exactly express my own view, although he interprets the change as the result of growth (Wucherung) of the follicle rather than its disintegration.

He says, p. 130, that in Salpa pinnata a most important and signifi-

cant change takes place in the follicular wall. This change is the growth (Wucherung) of the wall, which begins at the lower part of the embryo, where the wall becomes greatly thickened and consists of vacuolated tissue. The growth of the follicular wall is brought about by the great multiplication of its cells, which are set free and lose their connection with each other and change their form. These changes begin at the bottom of the embryo and extend upwards. The section which is represented in his Fig. 29, which corresponds to my Plate XIII, shows, in the upper part of the embryo, the unmodified tissue of the follicular wall, which consists of densely crowded polygonal cells. This tissue is found in only a small part of the section, however, and he says that, in the greater part, the follicle wall is greatly thickened and consists of an intercellular fluid, and of cells which move freely through it. That these cells are free and able to move is shown, as he points out, by their variability and their amœboid shape.

The outline of the somatic layer persists for a little while after the disintegration begins, as my Plate XIII shows, but the whole structure soon breaks down and disappears, and Salensky's account, as well as my own, shows that its identity as a tissue is quickly destroyed, although he says, p. 159, that we cannot speak of the disappearance of the follicle, since its cells proliferate and furnish the material for the formation of the mesodermal and endodermal structures.

He says, however, p. 129, that as the result of this growth the whole of the secondary follicular cavity (body cavity) becomes filled with wandering amœboid cells from the walls of the follicle, and that there is thus formed between the epithelial capsule (Ectodermkeim) and the organs of the embryo a mass of wandering cells, which he regards as equivalent to a mesoderm.

His account of the phenomenon in Salpa fusiformis, p. 352, is very similar, as he says that the cells of the peripheral layer of the follicle separate from each other and become star-shaped, while a homogeneous semi-fluid substance, which does not stain in carmine, appears between them.

It will thus be seen that his view of the fate of the somatic layer is almost identical with the account which I have given on page 44, although he believes that the fate of other follicular structures is very different.

SECTION 5.— *The Fate of the Follicular Lining of the Atrium and Gill Tubes.*

I have already fully described on page 29 the way in which the atrium and gill-slits are formed by the invagination, from the somatic layer of the follicle, of two tubes, which I regard as the follicular equivalents of two perithoracic tubes. I have also shown, page 45, that the follicular lining of the perithoracic structures ultimately breaks up into amœboid cells, which at first almost completely fill the atrium, although they gradually disappear as development progresses.

Salensky's account of the origin of these structures is very different indeed from mine, since he believes, page 140, that the cloaca of salpa is not a distinct chamber, but only a part of the pharynx; that the "gill" is formed by the meeting and union of two outgrowths from the walls of the pharynx; and that the primitive digestive cavity consists at first of two independent halves, separated from each other on the middle line.

It is only necessary to compare his figures with my own, especially his Plate XIV, Fig. 33, with my Plate XIV, in order to discover that he has mistaken the two gill tubes, g^{IV}, of my figures, for the digestive cavity, and that a complete series of sections would have shown him the atrium g''', at a higher level, and the true pharynx c, at a lower level, at the stage of his Fig. 33.

His account of the origin and fate of the inner lining of his, so-called, primitive digestive cavity is identical with my own, however, for he says that it is derived from the somatic layer of the follicle (Follikelwand), and that it breaks up into amœboid cells which are gradually used up as food and disappear.

He says, on page 131, that each of the separate halves of which the primitive digestive cavity at first consists, is open above, where it is fastened to the somatic layer (Follikelwand), and that through this opening a string of follicle cells runs downwards into the digestive cavity, to end in a mass of cells which, in part, form its inner lining and, in part, lie free within it. He says, page 131 and page 127, that these lining cells have no formative function, but that they are purely nutritive, and that they gradually become less and less abundant as they are converted into food.

So far then there is no very great discrepancy between Salensky's account of the fate of the follicle and my own.

He regards the disintegration of the somatic layer as growth, but he agrees with me that it does disintegrate, and he regards the gill-tubes as

the primitive digestive cavity, but he agrees with me that their lining is derived from the somatic layer of the follicle, and that it is used up as food.

SECTION 6.—*The Nutrition of the Blastomeres and the fate of that part of the Visceral Layer of the Follicle which penetrates between the Blastomeres.*

We come now to a most interesting and remarkable feature in the history of the salpa-embryo, the nutrition of the blastomeres; and we are here brought into direct opposition to Salensky, for my view of the fate of the follicle cells which push in among the blastomeres is totally and fundamentally different from his, although even here the difference is rather in the interpretation of the observations than in the observations themselves.

I have already given, pages 25 and 26, a brief outline of the way in which the blastomeres are nourished by the follicle cells, but the subject is so important that I must now discuss it more minutely, although the reader may find in a review of these pages a good introduction to the following account.

During the early stages in the migration of the follicle cells nuclear figures are found occasionally in the visceral portion, as is shown in Plate X, Fig. 9, but these soon disappear, and while the cells, or their nuclei at least, continue to multiply with very great energy, this takes place by direct division. Plate XLII, Fig. 1, is a greatly magnified drawing, made under a Tolles $\frac{1}{12}$ used as a homogeneous immersion objective with a long tube and *A* eyepiece, of a section of an embryo of Salpa hexagona in the series from which Plate XI, Fig. 1, was drawn. In both figures, 7 is the somatic layer of the follicle; 8 is the visceral layer, and 9 the blastomeres. The somatic layer stains more deeply in hæmatoxylin than the visceral layer, and this is even more true of their nuclei, although at this stage the visceral nuclei take more color than they do at later stages.

The most conspicuous peculiarity of the visceral nuclei is that they are elongated in lines which are radial to the subspherical embryo. They are irregularly pear-shaped, and in most of them the small end is towards the center of the embryo. Even at this stage the amount of chromatin is less than it is in the somatic nuclei, and it is concentrated, in a mass which stains very deeply, at the central pointed end. Near the

top of the figure some of these elongated nuclei are shown in various stages of division. These are found in every section, and at a later stage, Fig. 3, almost every nucleus is dividing, or is about to divide, or has just divided, and the visceral nuclei thus show a marked arrangement in pairs.

The energetic multiplication takes place by direct division, and as it goes on the chromatin is gradually exhausted, and the nuclei become more and more transparent and vesicular, as will be seen by comparing Figs. 1, 2, 3, 4, 5, 6 and 7, 8 of Plate XLII. At the stage shown in Figs. 1 and 2, the division takes place in planes concentric with the outer surface of the embryo, or at right angles to the radii and to the long axes of the nuclei, so that each nucleus falls, after the division, into a peripheral and a central portion, lying in the same radius.

The outlines of the visceral follicle cells, at this time and later, are so obscure that it is difficult to decide whether the division of the nuclei is, or is not, accompanied by cell division. Such cell boundaries as are visible are always radial, as Figs. 2 and 5 show, and I have found no trace of cell division in the planes of nuclear division, and it seems probable that nuclear division goes on more rapidly than, or perhaps without, cell division, so that each cell soon becomes polynuclear.

Salensky shows four of these pear-shaped nuclei, with a deeply stained area at the small end, in an embryo of Salpa democratica, near the top of his Taf. 27, Fig. 2, *dt*, but he does not figure them in any other species, and I can find no account of them in the text. We cannot fail to notice the resemblance between those shown in my Fig. 1, Plate XLII, and the pear-shaped bodies with a deeply stained spot at the small end, which are figured in pyrosoma by Salensky (15), especially in his Figs. 11, 12, 14 and 19, although he does not regard them as nuclei, but as pear-shaped follicle cells (Kalymmocytes), with the deeply stained nucleus at the small end. However the case may be in pyrosoma, the pear-shaped bodies in the embryo of Salpa hexagona are not cells, but follicular nuclei.

The account I have given of the way in which the nuclei multiply will show that the result of this process must be a migration inwards towards the center. Those which move in lines which carry them to the blastomeres push into the layer of protoplasm around the big spherical blastodermic nuclei. In several sections I have found follicular nuclei half imbedded in the protoplasm of the blastomeres, like the one which is shown on the left side of the middle blastomere in Fig. 1; and nuclei which retain their pear-like shape and the spot of deep color at the small

end are often to be seen in the protoplasm. One of them is shown near the middle of the left side of the lowest blastomere in Fig. 1. Besides these well marked and characteristic nuclei, the protoplasm of the blastomeres contains a number of vaguely defined indefinite bodies which stain diffusely and uniformly in hæmatoxylin. Some of these are almost invisible, while others retain enough of the character of follicular nuclei to show that they enter the protoplasm of the blastomeres in great numbers, and are gradually dissolved and lost in its substance. The fate of those which are not brought by their lines of motion into contact with the blastomeres is somewhat different. They lose their elongated shape and become spherical, their chromatin becomes diffused and they finally grow indefinite, and appear to break up at last into separate granules.

These phenomena seem to me to admit of only one interpretation. Some of the nuclei penetrate the substance of the blastomeres and are digested, while others degenerate and go to pieces among the blastomeres to supply them with food. During the early stages in the history of the blastomeres, most of the assimilated food seems to be converted into chromatin, for comparison of Figs. 1, 2, 3 and 4 shows that while the protoplasm of the blastomeres remains transparent, the nuclear network becomes more and more conspicuous, while the central nucleolus increases in size. As the blastomeres multiply and become smaller, Figs. 5, 6 and 8, their protoplasm also gradually becomes granular and opaque, so that they are distinguished from the surrounding follicle cells, both by their granular structure and by the nucleolus and chromatic network, as is shown in Fig. 9, where 8 and x are follicle cells, and b and a endodermal and ectodermal blastomeres.

So far as I am aware, no one except Salensky has noted the existence of the follicular nuclei in the protoplasm of the blastomeres, and his interpretation of them is directly opposed to mine, as he sees in them evidence of the degeneration of the blastomeres.

He believes that the tissues of the embryo are formed out of the follicle cells (gonoblasts, kalymmocytes), and that the blastomeres are used up as food, and on page 340 (5), he gives the following statement of his view: "All the organs of the body of the embryo are built up from gonoblasts, while the blastomeres pass more and more into the background and play scarcely any part in the formation of the organs. Development begins according to the general type of sexual reproduction, but it soon passes over into the asexual. Since the construction of the salpa-embryo proceeds from those elements which are not derived

from the fertilized egg, and since it presents many points of resemblance to asexual multiplication, especially to the process of budding, I have proposed the name follicular budding for this sort of reproduction. The fundamental characteristics of follicular budding, which have impelled me to the use of this name, are the proliferation of the follicle cells, and their contribution to the construction of the embryo."

My own observations seem to me to prove that the nuclei of many of the visceral follicle cells become converted into food for the developing blastomeres, while Salensky holds that the blastomeres degenerate and supply food for the follicle cells, but so far as our observations go there is no very essential disagreement.

In his account of the embryo of Salpa pinnata, at about the same stage as my figure of the embryo Salpa hexagona, Plate XI, Fig. 2, he says (5), page 99, " I will here call attention to the noteworthy changes in the histological condition of the blastomeres. Their protoplasm, which at all the earlier stages was finely granular and almost transparent, now falls into small, irregular patches, which are sometimes around the nucleus and sometimes at the periphery of the cells. When I first discovered this *peculiar degeneration* of the protoplasm, I attributed it to the action of the preserving or staining fluids. It is, however, so constant at certain early stages, after the first steps in segmentation, and is so independent of the method of preservation, that I am convinced that this alteration of the protoplasm is a normal phenomenon in the development of salpa. Briefly stated, the protoplasm falls into a central mass around the nucleus and a number of peripheral polygonal portions."

His statement, page 100, that while these changes are taking place in the blastomeres, the follicle cells (gonoblasts) present nothing noteworthy except their vigorous multiplication, shows that he failed to study the stages shown in my Plate XLII, Figs. 1 and 2, for at this time the changes in the follicular nuclei are eminently noteworthy.

His figures of the embryo of Salpa democratica are most instructive, and seem to prove, almost as clearly as my own, that the bodies in the protoplasm of the blastomeres are migrating follicular nuclei.

I have already said that in his Taf. 27, Fig. 2, *dt*, he has drawn four of the peculiar pear-shaped migrating nuclei, and in Taf. 27, Fig. 1, *dt*, *A*, he shows a great number of bodies of the same size and shape in the protoplasm of the blastomeres, and gives, on page 379, the following account of them: "The protoplasm of the blastomeres of Salpa democratica is, in contrast to that of other species, perfectly homogeneous. It is note-

worthy that the destruction of the protoplasm which takes place in all the other species, is not to be observed in Salpa democratica. In place of it, we find scattered in the protoplasm of the blastomeres *small, round bodies which color deeply in carmine.* I can give nothing more exact regarding their origin and significance, and will only add that these bodies are also found in the follicle cells around the blastomeres."

While he is thus in doubt regarding Salpa democratica, the passages which I have quoted show that in all other species he regards these bodies as products of the degeneration of the blastomeres; but his view is not very clearly stated, for on page 103 he says that they have a great resemblance to the deutoplasm of *other eggs*, with which they are perfectly analogous as regards their later history. I am not able to discover what it is which he compares with other eggs, for of course neither the blastomeres nor the follicle cells are eggs.

SECTION 7.—*The Fate of the Portion of the Visceral Layer which invests the Blastomeres.*

The origin of this layer of follicle cells is shown at *8* in Plate X, Figs. 3 and 5. Its cells elongate and multiply until it forms a thick wall around the visceral mass, as shown at *8* in Plate XLII, Figs. 5, 6, 7 and 8, and also in Plates XIII, XIV and XXII. After the blastodermic lining of the pharynx and perithoracic structures is formed, this layer lies between it and the body cavity, as shown in Plates XVI and XVII. It persists as a distinct tissue until the embryo is well advanced, but finally its cells become separated, as shown in Plate XVIII, and wander through the body cavity as migrating follicle cells. The disintegration begins at the bottom of the embryo and gradually extends upwards, and Plate XVIII, Fig. 7, *8*, shows some of these in the act of separation. After they become free they become vacuolated, as shown in Plate XX, Fig. 5, and the blood corpuscles, or mesenchyma cells, settle upon their surfaces and penetrate their substance, and form over them a network of fibers, Plate XXI, Fig. 2, which persists as a loose, honeycombed tissue, Plate XIX, Fig. 9, after the cells have entirely disappeared.

SECTION 8.—*The Placenta.*

I have shown (pp. 48–52) that the function of the placenta of salpa is quite unlike that of the mammalian placenta; that it is neither respi-

ratory nor excretory, but that it is purely a nutritive organ.   I have also shown that its cavity is a single chamber; that all its tortuous and irregular spaces open at last into the blood spaces of the chain-salpa; that the immediate function of the placenta is to nourish its own constituent cells from the blood which is driven into it by the heart of the chain-salpa; and that the embryo is nourished by wandering placenta cells which migrate into its body.

The internal structure of the fully developed placenta is complicated and puzzling, but it is much simpler at the younger stages which are shown in Plate XVIII, in the transverse sections in Figs. 3, 4, 5 and 6, and in the longitudinal sections in Fig. 8.   In Plate XLVI three horizontal sections, Figs. 2, 3 and 4, are shown at the stage which is shown, in longitudinal section, in Plate XXXV.   Its boundary wall, Plate XVIII, 23, is colored red in the figures, and is derived (p. 243) from the lower part of the epithelial capsule.   The roof, 10, is colored blue and is derived (p. 269) from the somatic layer of the follicle.   The cavity of the placenta is filled by a spongy mass of strings of cells, which are colored blue in the figures.   They are derived, as Fig. 3 of Plate XI and Fig. 1 of Plate XLV show, from the roof of the placenta.   This mass ends below in the so-called "blood-bud" or "bell-clapper," 24, which, as transverse sections show, Plate XVIII, Fig. 4, 24, divides the neck of the placenta into two channels which communicate with each other through the irregular spaces in the upper part of the spongy mass of cells.   These cells are nourished by the entangled blood, and they not only grow to a great size, but they also store up a yolk-like substance and thus come to resemble eggs.   Finally they push through the roof of the placenta into the body cavity of the embryo, where they are shown in Salpa pinnata at 29 in Plate XVIII, and in Salpa hexagona in Plate XLV, Fig. 4, 29.

The structure of the fully grown placenta is so complicated and irregular that it is necessary to study complete series of sections, in at least two planes at right angles to each other, to gain a clear picture of it, but serial sections prove that it contains only one chamber, and that this has no direct connection with the body cavity of the embryo, while it is directly continuous with the blood system of the chain-salpa.

I have shown, page 49, that a functional identity with the mammalian placenta has been generally assumed, and it is not at all strange that the authors who have studied it with this hypothesis in mind have misinterpreted the complicated passages in its upper portion, and have believed in the existence of the structure which its imaginary physiology has been supposed to require.

Thus Leuckart (1) says that its physiology is like that of the mammalian placenta, and that, in origin, it is, as it were, the remnant of the yolk left over as a yolk-sac after the embryo is formed, and that instead of being turned to use directly in the construction of the embryo, it is converted into a necessary organ of the fœtus. Its modifications have for their purpose simply to increase the surface by which the body of the embryo comes into contact with the current of maternal blood, and while it is at first solid, it soon becomes hollowed out into a cup-shaped cavity which communicates with the circulatory organs of the mother, and which is to be regarded as a maternal blood channel. Its inner wall, which is freely bathed by the blood of the mother, exhibits numerous irregular processes which, as a rule, run like ribs from the top of the placenta to its opening. Often there is a knob-like projection which hangs down from the roof of the cup to some distance into its cavity.

It will be seen that this brief sketch of its structure is accurate, and while Barrois and Salensky have shown that it is no way comparable to a yolk-sac, and that its chamber is not hollowed out of a solid rudiment, their more elaborate accounts of its structure are less correct than Leuckart's.

Barrois (4) says, page 495, that its function is like that of the mammalian placenta, and, page 484, that, when fully formed, it consists, in Salpa africana, of two separate chambers, a fœtal chamber and a maternal chamber. The lower chamber, which is identical with the cul-de-sac of earlier stages, and is therefore part of the blood system of the chain-salpa, is the maternal placenta. It opens below into the blood space of the chain-salpa, and its roof, which separates it from the upper or fœtal chamber, is derived from the lower part of the follicle, and it carries the "bell-clapper" pendant from its center. Its outer edges are continuous with the side walls of the placenta.

He says that the fœtal chamber is at first in communication with the digestive cavity of the embryo, and that its roof is to be traced back to a saddle-shaped layer of endodermal blastomeres, which, at a very early stage, separates from the embryo. In transverse sections this layer is found to be made up of long cylindrical cells with many nuclei which increase in length towards the top. These cells are identical with the "germoblastic cells" of Todarro. At their inner ends these cells soon begin to degenerate, and to give rise to the "yellow bodies" and "oviform bodies" of Todarro, which latter are derived from the tissues of the

embryo and ultimately from the egg, and not from the lateral walls of the placenta, which, as Barrois points out, are derived from the walls of the cul-de-sac, and are formed from the body of the chain-salpa.

He says that degeneration begins around the circumference of the membrane which forms the roof of the maternal chamber and the floor of the fœtal chamber, and that it takes place in such a way that this is set free from the inner surface of the wall of the placenta. The degeneration extends towards and finally reaches the central thickening, or "bell-clapper," which loses its distinct epithelial character and becomes an irregular mass of cells. At the same time there is an increase in the products of degeneration, which are formed from two sources: 1st, at the expense of the two great masses of elongated cells which form the wall of the placenta at the level of the superior portion of the fœtal chamber, and 2d, at the expense of the central portion of the folded membrane, at the middle of which there is heaped up above the "bell-clapper" a great accumulation of granules and protoplasm. The outlines between the cells in the degenerating area disappear, and for a time the nuclei can be seen scattered through the transparent mass which is produced by their fusion, but as degeneration goes on the whole becomes granular, with nuclei and oviform bodies.

The "roof of the fœtal chamber" of Barrois seems, from his figures and description, to be what I have called the roof of the placenta and have marked *10* in my figures; and the "folded membrane" which he regards as the floor of the fœtal chamber and the roof of the maternal chamber is the horizontal fold, which is shown just above the "bell-clapper," *24*, in my Plate XVIII, Fig. 4, although serial sections would have taught him that it runs through only a few sections, and does not form a partition, since there is a free channel both in front of it, Fig. 3, and behind it, Fig. 5. His "oviform bodies" are the big migrating cells, *29*, and his "great accumulation of degenerating cells and nuclei and granular protoplasm" is the spongy mass of strings of follicle cells which fills the upper part of the placenta.

Salensky's account of the placenta (5) is scattered through his pages in such a way that it is hard to follow. The placenta of Salpa pinnata is treated on pp. 102, 111, 123 and 384; that of Salpa africana, on pp. 155 and 157; that of Salpa punctata, on pp. 331, 332 and 344; that of Salpa runcinata (fusiform), on pp. 347, 349 and 350; that of Salpa bicaudata, on pp. 368 and 397, and that of Salpa democratica, on pp. 379, 380 and 384.

He says little about its function, and it is difficult to state his view of its structure and origin briefly, both on account of the manner in which he presents it, in detached sentences, which are nowhere combined in a general view, and also because he uses the word "placenta" in no less than three ways. In his account of Salpa africana, for example, and in many other places, this word is used to designate the whole organ, as I have used it. In other places, p. 102, p. 128 and p. 384, it is used to designate the parts derived from the epithelial capsule (Epithelhügel and Ectodermkeim), as distinguished from those derived from the follicle, while in other places, pp. 379 and 384, it is used to designate the central structures derived from the follicle as distinguished from the side walls derived from the epithelial capsule.

His account shows that its structure varies greatly in the different species, but his description conveys the impression that the difference is fundamental, although his figures show that this is not the case. Thus, for example, his account of the placenta of Salpa democratica, pp. 379 and 384, indicates that the whole placenta is formed from the central structures, and that side walls derived from the epithelial capsule (Epithelhügel), and comparable to what, in his account of Salpa pinnata, p. 102, he calls the "placenta," are absent in Salpa democratica, although his figures, especially Plate XXVII, Fig. 3, represent distinct side walls, which, in this figure, are marked *Eph*.

The big migrating placenta cells, *29*, of my figures, which have migrated from the roof of the placenta into the body cavity of the embryo, are represented in Salpa pinnata, in his Plate XIV, Figs. 37, *pin* and 37, *pin A*, but while they are such a conspicuous feature in its development, I can find no account of them in the text. They are also shown in the embryo of Salpa bicaudata in Plate XXVI, where they are marked *plz*. In his account of this species he says, p. 367, that they are placenta cells, and on p. 370, that they fuse together at a later stage and form a pad which takes the place of the roof of the placenta.

As regards the structure of the placenta, I believe that careful study of Salensky's memoir proves the correctness of my view that it is fundamentally different from that of mammals, that it contains only one chamber, which is a diverticulum from the blood system of the chain-salpa, and that it is purely a nutritive organ.

His account of it in Salpa pinnata, pp. 102, 111 and 123, agrees in all essentials with my own observations, and he shows that there is here only one chamber; that the central structures and part of the roof are

derived from the follicle, and that the side walls and part of the roof are formed from the lower part of the epithelial capsule or "Ectoderm-keim," which becomes thickened, and, losing its connection with the part which invests the embryo, bends inwards towards the axis of the placenta.

He says, p. 157, that while the roof of the placenta of Salpa africana is formed from follicle cells, and not, as Barrois states, from blastomeres, his own studies of this species completely confirm all of Barrois' chief results.

I have shown, however, that Barrois has mistaken a transverse rod for a complete horizontal diaphragm; and that the division which he describes, into a fœtal chamber and a maternal chamber, is the result of this error, and that his figures show that there is really only one chamber in this species.

Salensky says, pp. 347, 349 and 350, that the placenta of Salpa runcinata (fusiformis) is very similar in its changes of form to that of Salpa africana, although it arises in a different way. It is at first cup-shaped, but it soon divides into two parts, an upper one which forms part of the body of the embryo at the time of its escape, which he calls the fœtal placenta, and a lower one which remains in the body of the chain-salpa, and which he calls the maternal placenta.

The study of his figures will show, however, that there is no division into two chambers, and that the only reason for calling the upper part a fœtal placenta is, that it is carried off by the embryo to be used up as food after its escape. His figures of Salpa runcinata and Salpa democratica show only one chamber, in direct connection with the blood system of the chain-salpa, and while, in the absence of any figures of transverse sections, his longitudinal sections of the placenta of Salpa bicaudata are almost unintelligible, they do not indicate any difference from the other species in this particular.

I think that we may affirm, then, that no species of salpa has been proved to have a fœtal placenta, and that all the evidence indicates that it is in all cases a single chamber, in communication with the blood system of the chain-salpa.

SECTION 9.— *The " Test Cells " of Salpa.*

I have shown, page 220, that the follicle cells of salpa must be regarded as germ cells which have become differentiated from the

reproductive cells and have been specialized for another function. I have also shown that this function is nutritive, and that they all become food for other cells and tissues. The follicle cells have great vegetative power, and while some are used up as food very early, others continue to grow and multiply until the embryo has reached an advanced stage of development, although ultimately their fate is the same.

I have shown also that while some of them, page 271, wander through the body cavity of the embryo as amœboid cells before they become vacuolated and disappear, others, or their nuclei at least, page 273, push into the substance of the protoplasm of the blastomeres, during the later stages of segmentation, and supply them with food, while still others, page 223, enter the substance of the ovarian eggs, before fertilization, and furnish the food which they assimilate and turn into yolk.

These latter cells, the follicle cells which migrate into the ovarian ova, are "test cells," and as their history is only a small part of the history of the migrating follicle cells of salpa, the extension of the period of migration through the stages of segmentation into the embryo, and its persistence up to a period when the embryo is well advanced in its development, give us a new basis for discussing the nature of "test cells."

I have no desire to enter the interminable "test cell" controversy, nor to make a new addition to its voluminous literature, and I shall not venture to open anew the dispute which has already exhausted the patience of embryologists, although I believe that the life-history of salpa furnishes evidence of an entirely new sort.

The history of the discussion up to the year 1889 may be found in Davidoff's memoir on Distaplia (16, pp. 138–190), and it is not necessary to review it again.

The wandering cells which I have described among the young ova of salpa are "test cells" identical in their origin with those which Ulnanin (pp. 7, 40) has figured and described in the young egg of doliolum, and the history of the later stages in the life of the egg and embryo of salpa proves that they are follicle cells, and that their appearance is the first stage in the follicular migration which afterwards becomes so prominent.

Whether the "test cells" of all tunicate eggs are migrating follicle cells or not, is a question upon which I shall express no opinion.

CHAPTER XIV.

THE ONTOGENY OF THE ORGANS OF SALPA.

Section 1.—*The Salpa Embryo.*

As I have already stated, the most remarkable peculiarity of the salpa embryo is this: It is blocked out in follicle cells which form layers and undergo other changes which result in an outline or model of all the general features in the organization of the embryo. While this process is going on the development of the blastomeres is retarded, so that they are carried into their final positions in the embryo while still in a very rudimentary condition.

Finally, when they have reached the places which they are to occupy, they undergo rapid multiplication and growth, and build up the tissues of the body directly, while the scaffolding of follicle cells is torn down and used up as food for the true embryonic cells.

No other animal presents us with an embryonic history quite like that of salpa, although other tunicata show something similar, but very much less pronounced. In the chapter on the morphological significance of the salpa embryo, I attempt to show how the life-history of salpa has come about, but we must now confine ourselves to the facts.

An imaginary illustration may help to make the subject clear. Suppose that while carpenters are building a house of wood, brickmakers pile clay on the boards as they are carried past, and shape the lumps of clay into bricks as they find them scattered through the building where they have been carried with the boards. Now, as the house approaches completion, imagine that bricklayers build a brick house over the wooden framework, not from the bottom upwards, but here and there, wherever the bricks are to be found, and that, as fast as parts of the brick house are finished, the wooden one is torn down. To make the analogy more complete, however, we must imagine that all the structure which is removed is assimilated by the bricks, and is thus turned into the substance of new bricks to carry on the construction.

Salensky (Neue Untersuchungen, etc., Naples Mittheilungen I, 1882, and Embryonalentwicklung der Pyrosoma, Zool. Jahrbücher, IV and V,

1891) has discovered and minutely described the migration of the follicle, but he has failed to trace the history of the blastomeres, and believes that these degenerate and disappear, and that the embryo is built up of follicle cells. I find that all the follicle cells are ultimately used up as food, and that the true embryo is formed from blastomeres after the analogy of the rest of the animal kingdom.

SECTION 2.—*The Aggregated Salpæ.*

During their development the aggregated salpæ undergo complicated changes of position, which render the interpretation of sections very difficult, and as both Salensky (Morph. Jahrbuch, 1877, III) and Seeliger (Jena. Zeitsch., 1885) have totally failed to understand these changes, their accounts of the origin of the aggregated salpæ have no permanent value.

I pointed out, in 1886 (Studies from the Biological Laboratory, Johns Hopkins University, 1886, pp. 398–414), that the salpa-chain is, morphologically, a single row of salpæ, all in the same position, with their dorsal surfaces proximal or towards the base of the stolon, and their right sides on its right. The account of the origin of the aggregated salpæ, which is given in this memoir, is simply an amplification and expansion of the statement which, in 1886, I made briefly and in outline.

The stolon is bilaterally symmetrical, its plane of symmetry is fundamentally identical with that of the solitary salpæ, and the rudiment of each aggregated salpa is bilaterally symmetrical in the same plane, although the secondary changes begin very early and convert the single row into a double row, which comes to consist of a series of right-hand salpæ and a series of left-hand ones placed with their dorsal surfaces out, their ventral surfaces towards the ventral surfaces of those in the opposite row, and with the left sides of those on the right, and the right sides of those on the left towards the base of the stolon. In order to illustrate these secondary changes of position let us represent the series of salpæ by a file of soldiers all facing the same way. Now imagine that each alternate soldier moves to the right, and the others to the left, to form two files still facing the same way. Now let them face about so that the backs of those in one row are turned towards the backs of those in the other row. They will now represent two rows of salpæ in their secondary positions.

To make the illustration more perfect, suppose that, instead of step-

ping into new places, the soldiers grow until they are pushed out by mutual pressure, and suppose that their heads, growing fastest, form two rows while their feet still form one row, and suppose, furthermore, that, as each soldier rotates, his feet turn first, and that the twisting runs slowly up his body to his head which turns last. We must also imagine that these various changes all go on together, and that while they are taking place each soldier not only grows larger, but also develops from a simple germ to his complete structure.

Salensky regards the stolon as two rows of rudimentary salpæ, and while Seeliger correctly states. that they first form a single row, he has failed to discover the rotation, and believes that they arise on the stolon in their final positions, and he has, therefore, failed as completely as Salensky in his efforts to trace the origin of their organs.

SECTION 3.—*The Ectoderm of the Salpa Embryo.*

I have already, page 40, described the origin of the ectoderm of the salpa embryo. Briefly recapitulated, its history is as follows:

At an early stage of segmentation, some of the blastomeres, Plate XI, Fig. 1, *9'* and Fig. 4, *9'*, move upwards and pass out of the follicle on the middle line of the dorsal surface where the two layers are continuous with each other. I have given on page 63 my reasons for believing that this is the spot which was once occupied by the blastopore.

The ectodermal blastomeres, Plate XVII, Fig. 5, *9'*, thus become extra-follicular, although they are covered for a time by the epithelial capsule, *b'*. They give rise by cell division to the ectoderm, Plate XLII, Fig. 11, *a*, which spreads from the dorsal middle line outwards and downwards over the embryo, pushing off and replacing the cells of the epithelial capsule, Plate XVII, Fig. 1, *b'* and Fig. 6, *b'*.

The ectoderm has a growing edge, Plate XVIII, Figs. 4, 5 and 6, like that of meroblastic embryos, page 57, and it does not close in completely on the ventral middle line until after birth.

Salensky has figured the migration of blastomeres to an extra-follicular position on the dorsal surface of the embryo in several species, although he seems to regard them as discarded blastomeres, and derives the ectoderm from other sources.

They are clearly shown in Salpa pinnata, in his Plate XII, Fig. 26; in Salpa pectinata, in his Plate XXIII, and in Salpa fusiformis, in his

Plate XXIV, Fig. 3, where they are marked by the letters *Eckb*, which might be supposed to stand for "ectodermal blastomeres," if he did not tell us explicitly on page 389 that the ectoderm of this species is derived from the epithelial capsule (Epithelhügel).

The ectodermal blastomeres seem to be more conspicuous in Salpa fusiformis than in other species, for Salensky says, page 347, that while the epithelial capsule (Ectodermkeim) is generally separated very sharply from the embryonic cell mass, it is, at one end of the embryo, so intimately related to the follicle cells (gonoblasts) that it is difficult to determine the boundary between them; *and the blastomeres which lie directly at this spot* are covered only by the cells of the epithelial capsule (Ectodermkeim). At a later stage, page 350, he says that the epithelial capsule (Ectodermkeim) contains cells which differ greatly among themselves in both size and form. Some of these are similar in appearance to the cells of the epithelial capsule as already described at an earlier stage, and differ from them only in being flattened. "*The others (Eckb) are very much larger and very different in structure. They stain only slightly in carmine, and contain a nucleus which is very similar to that of the blastomeres. The appearance of these cells suggests that they are blastomeres which have passed out from the cell mass.*"

Salensky believes that the ectoderm of Salpa democratica is derived from the oviduct, and that in all other species it is derived from the epithelial capsule, but I think all will agree that his position is untenable until he has traced the history of the extra-follicular blastomeres, and has proved that they take no part in its formation.

I have shown, however, that they do give rise to the ectoderm, and that the epithelial capsule is a transitory structure which is lost as the ectoderm replaces it.

SECTION 4.—*The Ectoderm of the Stolon and that of the Aggregated Salpa.*

All observers agree that the ectoderm of the stolon is derived directly from the ectoderm of the embryo, substantially as I have described it on page 68.

In one minor point my own observations have shown that the older accounts are inaccurate. It is usually stated that the ectoderm of the embryo becomes pushed out into a tube by the growth of the other constituents of the stolon, and Seeliger says (11), p. 13, that it is an evagination (Ausstulpung) from the ectodermal epithelium of the embryo.

This is not literally true, for the ectoderm itself takes a most active part in the formation of the stolon, as I have shown on p. 68. This is marked off on the body of the embryo by a fold of ectoderm which pushes backwards from its tip to its base, so that it is folded off from the body of the embryo rather than pushed out, and in the younger stages of Salpa pinnata at least, its differentiation from the body of the embryo is chiefly due to the active growth of this ectodermal fold, as I have shown on p. 68 and in Plate XX, Figs. 5, 6 and 7, Plate XLI, Figs. 4 and 6, and Plate XVI, Fig. 5.

The ectoderm of the aggregated salpa has been correctly held to be directly derived from the ectodermal tube of the stolon by all students except Todarro. My own observations also show that the multiplication of the ectoderm cells is the chief active agent in the segmentation of the stolon, as I have stated on p. 78. This is exhibited by the following series of figures, Plate XXXIV, Figs. 8, 9, 10, 11, Plate XXIV, Fig. 5, Plate XV, Figs. 11 and 12, and Plate XXIX, Figs. 3, 4, 5, 6, 7 and 8.

These figures show that the nerve tube and the perithoracic tubes are cut up into vesicles by the growth of the ectodermal folds, and that these are the chief agents in the segmentation of the endodermal tube and of the genital string.

SECTION 5.—*The Nervous System of the Salpa Embryo.*

The salpa embryo is so very unfavorable for minute study that we cannot hope to do more than to show that the history of its organs is not inconsistent with our knowledge of the other tunicates. We must not look to it for contributions to comparative embryology, and I have little to add to the outline of the history of its nervous system which I have given on p. 37 and p. 41.

At the stage which is shown in Plate XII and in cut B on p. 29, the middle of the dorsal surface is occupied by the ectodermal blastomeres, A', and at each end of the body these may be traced downwards into the visceral mass.

Those at the posterior end are shown at *16* in Plate XII, Fig. 1, at *9* in Fig. 2, and at *18* in Figs. 3, 4 and 5. I regard them as the blastomeres of the caudal portion of the nervous system. They are inclosed in a mass of follicle cells which lies behind a second mass of blastomeres and follicle cells, Fig. 4, *19*, which I regard as the rudiment of the notochord. The blastomeres of both these regions soon become undistinguishable,

although at the stages shown in Plates XIII and XIV the follicular rudiment of the notochord, *19*, and that of the nervous system, *30*, are still distinct from each other. At the stage which is shown in Plate XVI and Plate XVII, however, this region of the body is occupied by a single mass of vacuolated and degenerating cells, the eleoblast, *k*, the later history of which has already been described on page 38. It seems to represent both the chorda rudiment and the nerve rudiment of the younger stages, and I have already shown, pp. 38 and 128, that it is homologous with the tail of ascidians and doliolum.

The blastomeres which extend downwards at the anterior end of the embryo, *3*, in Plate XII, Figs. 1, 2, 4, 5 and 6, form the rudiment of the ganglion which is represented in all the plates by *s*. It is enclosed in a mass of follicle cells, which are at this stage continuous above with those of the somatic layer of the follicle, while below they are continuous with the follicle cells of the visceral mass. It is probable that its connection with the somatic layer is primary, and with the visceral layer secondary, for in older embryo of Salpa pinnata it loses its connection with the somatic layer, while it at the same time comes into more intimate relation to the visceral layer, as Plates XVI, XVII and XVIII show.

In an embryo of Salpa hexagona, at about the stage shown in Plate XII, it is distinct from the visceral mass, and so intimately united to the somatic layer that, as Plate XI, Figs. 4, 5 and 6, *s*, show, its follicular portions seem to be an invagination from the somatic layer, *7*.

In ordinary tunicates the ganglion consists of two structures in intimate relation to each other—the ganglion proper, which is formed by the invagination of the dorsal ectoderm, and the so-called sub-neural gland, which is a diverticulum from the pharynx.

The connection between the follicle cells of the ganglionic rudiment and the somatic layer of the follicle is easily intelligible as a record of the primitive origin of the ganglion from the ectoderm.

After the ganglionic rudiment has lost this connection and has united with the visceral layer, the cavity of the pharynx becomes produced into it, as is shown at *s* in Plate XVIII, Figs. 2, 3 and 8. In Fig. 1 of Plate XVII the pharynx consists of two parts, a very broad portion which lies under the perithoracic tubes, and a much narrower part which runs forwards and meets the ectoderm at the point where the mouth is afterwards formed. The cavity of the ganglionic rudiment opens into the roof of this narrow oral division of the pharynx.

In older embryos, Plate XXXV, the ganglion, *s*, and sub-neural gland are clearly comparable with those of ordinary tunicates.

SECTION 6.—*The Nerve Tube of the Stolon.*

The straight stolon of Salpa pinnata is so favorable for studying the origin of the nerve tube, and the evidence of its ectodermal origin is so simple and clear in this species, that it hardly seems worth while to devote much space to the discussion of observations which have been made upon twisted stolons like that of Salpa democratica, where it is very difficult to study the young stolon by sections. The connection between the nerve tube and the ectoderm, as I have described it on p. 70, is shown for only a short time, however, in very young stolons, and older stolons furnish no evidence whatever as to its origin.

Of the various writers on the subject, Kowalevsky (Beiträge zur Entwicklungsgeschichte der Tunicaten, Nachrichten der königl. Gesellschaft der Wissenschaften zu Göttingen, 1868, 19) seems to regard it as mesodermal in origin. Salensky (Ueber die Knospung der Salpen, Morph. Jahrb., 1877, Bd. 111) says nothing about its origin. Todarro (Sopra lo sivelluppo e l'anatomia delle Salpe, 1875) derives it, as he does all the other organs of the stolon, from a single germoblastic cell; but I have already shown that his germoblastic cell is a migrating placental cell, and all recent writers have justly rejected his account of the stolon. Seeliger (11 and 15) believes that in the stolon of salpa, and also in the buds from the ascidiozooids of pyrosoma, it is mesodermal in its origin, and that it is derived from an indifferent mass of mesoderm, which, in the young stolon, fills all the space between the ectoderm and the endodermal tube, and becomes differentiated into the nerve tube and the other organs of the stolon. I have not found at any stage anything in the straight simple stolon of Salpa pinnata corresponding to his indifferent mesoderm, although I have studied it in serial sections at all stages in the three rectangular planes, and I do not hesitate to affirm that Seeliger has been misled through the selection of a most unfavorable species of salpa. As I have not myself studied pyrosoma, I am not in a position to make any comment upon his account of this animal, although Salensky (17) has recently shown that the ganglia of the four ascidiozooids which are produced from the stolon of the cyathozooid, as well as the ganglion of the cyathozooid itself, are derived from the ectoderm.

SECTION 7.—*The Ganglia of the Aggregated Salpæ.*

The nerve tube arises as a solid rod, Plate XX, Fig. 4, *l*, but it soon acquires a lumen, Plate XXI, Fig. 7, *l*. As the ectodermal folds grow inwards and mark out the bodies of the salpæ, they cut the tube up, Plate XV, Fig. 10, *s*, into a series of ganglionic vesicles, one for each salpa, with cavities which are segments of the lumen of the tube.

The presence of the ectodermal folds and the growth of the ganglion soon cause crowding and pressure, and the ganglia, Plate XV, Fig. 10, *s*, and Plate V, Fig. 1, *s*, become flattened in the axis of the stolon and elongated transversely. As the oral ends of the pharyngeal pouches, Plate XXIV, Fig. 5, *27* and *28*, grow up to the level of the ganglion, and push in between it and the ectoderm, as shown in cut O on page 81, the crowding becomes still greater, and the single series of ganglia breaks up into two rows, which move to the right and the left alternately, as shown at *s* in Plate XXXI, Figs. 1, 2 and 3, *B* and *C″*.

I have already shown that when the salpæ are in their primary or morphological positions the dorsal surface of each is turned towards the root or proximal end of the stolon and its ventral surface towards its tip. In the series of figures on Plate XV and Plates XXIII and XL, all the sections are figured with the proximal end of the stolon at the bottom, so that, so long as the primitive position is retained, the dorsal surface of each section is below. Most of the reconstructions of the transverse sections on Plates V to VIII are proximal views like the diagrams on pp. 81 and 82.

As the pharyngeal pouches push in behind the ganglion they meet and unite on its ventral or distal side, Plate XXXI, Fig. 3, *B–B′*, so that, from this time on, the ganglion occupies its normal position above the dorsal middle line of the oral end of the pharynx, and its changes of position with reference to the axis of the stolon, which are shown at *S* in Plate V, Figs. 1, 2, 3, 4 ; Plate VI, Figs. 1, 2, 4 ; Plate VII, Figs. 3, 4, 5, and Plate VIII, Fig. 1, are due to secondary changes in the position of the middle plane of the body of the salpa as already explained, page 109, and not to any change in the relation of the ganglion to the other organs of the body.

It is convenient, however, to describe the ganglion as "migrating" outwards from the middle line to the right in a right-hand salpa, Plate V, Figs. 1, 2 and 3 ; and to the left in a left-hand one, Fig. 4, and moving downwards, Plates V, VII and VIII, until, in Salpa pinnata, it comes

to lie far below the level of the stolon. For a long time the ganglion retains its proximal position in Salpa pinnata, Plate VI, Fig. 4, s, and Plate XXXIII, Fig. 7, s, and this position is retained for a still longer time in Salpa cylindrica, Plates VIII, Fig. 2, s, and XL, Fig. 14, s and 19, s, but it ultimately moves outwards away from the axis of the chain, as Plate VIII, Fig. 1 and Plate XXXVIII, Fig. 41, show. I must repeat that there is no real migration of the ganglion; that it occupies its true position with reference to the body of the salpa at all stages from the first, Plate V, Fig. 1, to the end, and that its apparent migration is due to changes in the position of the body of the salpa. At all stages it lies in the middle plane, dorsal to the pharynx, at the end of the body, and thus furnishes a conspicuous index to the changes in the position of the middle plane.

Seeliger has correctly described the segmentation of the nerve tube and the "migration" of the ganglion, which he erroneously regards as an actual change in its position with reference to the other organs of the body. He also believes that the ganglionic rudiment gives rise, not only to the ganglion, but to the ciliated groove or sub-neural gland, although he admits that his observations upon this latter point are not conclusive (11), page 70. My own observations show clearly that the ciliated groove is not, in origin, part of the ganglion, but an independent outgrowth from the roof of the pharynx. It is shown at a young stage when it ends blindly, and has no connection with the ganglion in Plate VI, Fig. 4; Plate VII, Fig. 2, and in Plate XXXVI, Fig. 10, v, and Fig. 12, v.

SECTION 8.—*The Perithoracic Tubes and the Atrium, or Cloaca of Salpa Embryo.*

The history of the perithoracic structures of the salpa embryo has been described on pp. 29–35 and on pp. 123 and 124, and I have shown that while they are completely outlined in follicle cells before their blastodermic epithelium is acquired, the course of their development is intelligible only on the hypothesis that they are homologous with the corresponding structures of other tunicates.

Salensky has described the origin of the "gill" and of the median atrium or cloaca of the salpa embryo in a number of species, but the reader of his papers may search in vain for any basis of comparison

with other tunicates, or even for any fundamental unity in his accounts of the various species of Salpa, and his papers contain internal evidence that he has misinterpreted his observations.

Uljanin holds (7), p. 58 and p. 57, that the perithoracic structures of doliolum are not homologous with those of the ascidians, but I have already shown, page 130, that this view is not proved by his account of their development, even if we admit that he has traced their whole history in doliolum.

Salensky believes that the cloaca and "gill" of salpa are not homologous with the similar organs of the ascidians. He says that the "gill" is part of the body cavity which is shut in by folds in the walls of the pharynx, and that the cloaca is not an independent chamber, but a part of the pharynx which is separated off by these folds. A careful study of his description, especially pages 119, 200, 224, 225, and 229 of his first paper (2), and pages 114, 139, 160, 163, 338, 339, and 354 of his second paper (5), shows that his views not only involve this conclusion, but that, if accepted, they would also force us to believe that the "gill" and cloaca of one species of salpa are not homologous with the same structures in another species; for his account of their origin in Salpa democratica and Salpa pinnata has almost nothing in common with his account of them in Salpa africana, Salpa pectinata and Salpa fusiformis.

In his first paper on Salpa democratica, he says that, like Leuckart, he regards the gill as part of the inner mantle or branchial sac; that in origin it is nothing more than a strongly developed ridge or thickening on the middle line of the dorsal surface of the pharynx, and that, on each side of it, the cavity of the pharynx is pushed upwards to form a pair of pouches which soon meet and unite above the gill to form the atrium. In this way the gill ridge is transformed into a rod, and the rod, which is at first solid, becomes tubular by the conversion of its axial cells into blood corpuscles.

In this account of the origin of the perithoracic structures of Salpa democratica, the only point of agreement with my own observations on Salpa pinnata is his statement that the gill is at first solid, and that its central cells are set free as development progresses.

In his second paper (5), page 139, he retracts this statement, and says that while his studies of the embryo of Salpa pinnata have in other respects confirmed his account of the origin of the atrium and gill of Salpa democratica, they show that the gill is in its origin a hollow diverticulum from the body cavity.

He describes the atrium and "gill" as arising in a totally different way in Salpa africana, pp. 160 and 163; Salpa pinnata, pp. 338–39, and Salpa runcinata (fusiformis), p. 354, for while he says that in these species, as in Salpa democratica and Salpa pinnata, the atrium is part of the pharynx, it is separated off by two folds, Taf. 24, Figs. 7 and 8, *Kestp*, of its lateral walls, which grow towards each other and unite on the middle line to form a horizontal diaphragm, which shuts off the dorsal division of the pharynx from the ventral chamber. This diaphragm then becomes separated at its sides from the lateral walls of the pharynx, so that a secondary communication is established on each side between the upper and lower chamber, while the middle portion of the diaphragm becomes the gill. It will be seen that according to this account the "gill" of Salpa democratica and Salpa pinnata arises as an unpaired, median, dorsal fold, while in the other species it arises as a pair of lateral folds; in the first two species the gill-slits, or openings by which the two chambers communicate at the sides of the gill, are primary, while in the others they are secondary; in the first two the atrium is a secondary chamber formed by the union of two pharyngeal pouches, while in the other species it is primary.

The reconciliation of these statements with each other seems impossible, and any attempt to bring all or any of them into accord with my own account seems hopeless. More critical examination will show, however, that his observations are rather imperfect than inaccurate, and that his errors are errors of interpretation.

My own observations show that the perithoracic tubes and atrial chamber are formed before the cavity of the pharynx is hollowed out in the mass of visceral follicle cells; and Salensky has, in these early stages, mistaken them for the pharynx or "primitive digestive cavity." This is well shown by the comparison of the two figures of longitudinal sections of embryos of Salpa runcinata, which are shown in his Plate XXIV, Fig. 4 and Fig. 9. These figures show clearly that the so-called "Darmhohle," *Pmd*, of the younger embryo is the atrium, and not the pharynx, of the older one. This is proved even more conclusively by comparing his Fig. 11 of Plate VIII with Fig. 5; for the chamber which is marked *Pdmh* in the younger embryo is obviously the one marked *Kl* in the older one.

The perithoracic tubes are actually shown in many of his figures, notably in his Plate 6, Fig. 5, *pin*, where they are marked *Drm*. They are also shown in his Plate 12, Fig. 24, at *Dh*, and their union on the

middle line to form the atrium is shown at *D* in his Fig. 25, and at *D* and *Dh* in his Fig. 28, *A*.

In the series of sections in his Plate 13, Fig. 31, *A* shows the two perithoracic tubes cut above the level of the atrium as in my Plate XIII, Fig. 6, and in the diagram on page 30. His Figs. 31 *B* and 31 *C* show the atrium as in my Plate XIII, Fig. 7, and his Fig. 31, *D* shows the two tubes below the level of the atrium but above the level of the pharynx, as in my Plate XIII, Fig. 8.

Salensky regards these structures as the halves of the primitive digestive cavity which, he says, p. 114, arises in Salpa pinnata as two independent and completely separated halves, and he describes the atrium and gill as arising at a very much later stage in the way which is represented in his Plate XIV, Fig. 37, and Plate 15, Fig. 39.

In his Plate 24, Fig. 1 appears to be a section through one of the perithoracic tubes, *Pmdh*, before it has lost its connection with the surface, and in his description of this figure (5), p. 347, he says that the triangular primitive digestive cavity is united above to the epithelial capsule (Ectodermkeim), and on both sides of the tip are the reflections of the somatic layer (follicle cells) already noted, where this passes on into the visceral (gonoblastic) layer.

I have shown already, in the chapter on the follicle, p. 258, that Salensky correctly describes the manner in which the perithoracic structures (primitive digestive cavity) acquire their epithelial lining by the invagination of the somatic layer (follicular wall), and I believe that I have now carried the analysis of his observations far enough to prove that they contain internal evidence of the correctness of my own account.

Section 9.—*The History of the Perithoracic Structures of the Aggregated Salpæ.*

I have nothing to add to my account of the origin of the perithoracic tubes of the stolon on p. 75, or to my account on p. 79 of the way in which they are cut up into perithoracic vesicles by the folds of ectoderm. There are two of these vesicles in each salpa, and they give rise to the perithoracic system and to nothing else. Throughout its whole history the perithoracic system is bilaterally symmetrical, although this symmetry is hidden by the changes which take place in the position of the plane of symmetry during the growth of the embryo.

In my account of its history, p. 95, I have divested it of these secondary changes and have described it as it would be if there were no secondary changes of position, and we have now to consider its actual history as exhibited by sections.

As the right and left pharyngeal pouches, Plate V, Fig. 1, *27* and *28*, and Plate XV, Figs. 6, 7, 11 and 12, *27* and *28*, are formed, the perithoracic vesicles, *g* and *h*, are folded inwards by the growth of the ectodermal folds, so that each one of them lies on the dorsal or proximal surface of its corresponding pharyngeal pouch.

While the vesicles are hollow from the first, Plate XLIV, Fig. 10, *n*, they have at first no communication with the cavities of the pharyngeal pouches. The first trace of a gill-slit is a fold in the dorsal wall of the pharyngeal pouch, shown in Plate XXIV, Fig. 1, *A*, at *27* and at *28*, in salpa *C'*. This fold or outgrowth from the pharyngeal pouch elongates and soon unites with the perithoracic vesicle, *n*, to form a gill-slit, Plate XXV, Figs. 5, 6 and 7, *G-G'* and *H-H'*, at *g* and *h*. It will be seen that the endoderm is the active agent in the formation of the slit, and it will also be seen that the union between the perithoracic vesicle and the pharyngeal pouch takes place much earlier than the union of either of these structures with its fellow.

Soon after the gill-slits are formed the posterior ends of the bodies of the salpæ begin to push out to the right and the left, as Plate XXV, Figs. 6 and 7 show. The effect of this change is to convert the elliptical cross-section of the body into a wedge with its narrow edge on the left side of a right-hand salpa, *H-H'*, and on the right side of a left-hand salpa, *G-G'*. The two perithoracic vesicles are differently affected by the change, for while the one nearest the pointed end of the wedge is compressed in the line of the axis of the stolon, the other one is not. Thus the left perithoracic vesicle of the right-hand salpa, *n*, of *H-H'*, and the right-hand one, *g*, of the left-hand salpa, *G-G'*, becomes flattened and elongated towards the middle line, while the other, *g*, of *H-H'*, and *h*, of *G-G'*, remains more nearly circular in section.

Their relations to the morphological middle plane are fundamentally identical, but as the middle plane is itself moving outwards, there is an apparent asymmetry which the figures explain more clearly than words. Each perithoracic vesicle now becomes extended towards the middle line, where they unite to form the median atrium or cloaca. They contribute to it equally, and at the stage shown in Plate XXXIII, Fig. 3, they are shown at *g* and *h* at *N-N'* for a right-hand salpa, and at *K-K'* for a left-

hand salpa in contact with each other on the middle line but not yet united. These figures show how easily the study of transverse sections of the stolon at right angles to these might lead to the erroneous belief that the median atrium of a right-hand salpa is formed from the left vesicle alone, and that of a left-hand salpa from the right one alone. Plate VI, Fig. 1, is a proximal view of a right-hand salpa constructed from a series of sections at a stage a little younger than those last referred to. The right perithoracic vesicle is shown at g, the left at h, and the atrial prolongation of the left at g'''. The corresponding portion of the right one points towards the eye of the observer, and is, of course, indistinguishable. Fig. 2 of Plate VI shows the proximal surface of two salpæ, a right-hand one and a left-hand one, after the median atrium is formed; g in each is the right perithoracic vesicle, and h the left one, while g''' in the right-hand salpa is the portion of the median atrium which is derived from the left vesicle, h; and g''' in the left-hand salpa is the portion derived from the right vesicle, g. It is clear from the description that the middle line of the median atrium is in each salpa near the outer edge of the figure, over g, in the right-hand salpa, and over h in the left one. The rotation of the body soon carries the whole of the atrium out on to the external surface, as is shown at g''' in Plate VII, Fig. 4, and Plate XXXVI, Figs. 4, 5, 6 and 7; and it unites with the ectoderm at g^v on the middle line of the dorsal surface, and acquires a secondary opening to the exterior at g^v, Plate VII, Figs. 88 and 97.

Seeliger's account of the perithoracic structures of Salpa democratica (11), pp. 18, 48 and 63, serves to show how difficult the study of a simple structure may be made by a slight change of position, for phenomena which can be observed with ease in Salpa pinnata are so obscure in Salpa democratica that all the industry and technical skill which Seeliger has devoted to this species have had very little outcome. His account of the history of the perithoracic system is essentially as follows: The perithoracic tubes, which he calls the Seitenstränge, are mesodermal in their origin, and are specialized out of a mass of mesoderm cells which give rise also to the nerve tube of the stolon and to the genital rod. This mesoderm passes into the stolon from the body of the embryo in an unspecialized condition, and gradually becomes differentiated into these organs after the stolon is formed. The folds in the ectoderm of the stolon divide the "Seitenstränge" into a series of solid masses at the sides of the stolon between the ectoderm and the endoderm. These bodies are

equal in number to the future salpæ, and not twice as numerous. As each salpa is constricted off from the tube, it carries with it the greater part of one of the masses of cells from one side of the stolon, and the lesser portion of the one on the opposite side. These two masses are not bilaterally placed in the body, but are on the middle line, the larger one being dorsal or neural, and the smaller one ventral or hæmal. The latter gives rise to the heart and to the eleoblast, while the larger one on the neural surface gives rise to most of the mesoderm of the chain-salpa, and also to a cloacal vesicle, which is median and unpaired. This vesicle becomes distended, and at two points, one on each side of the middle line, it unites with the wall of the branchial sac, and the atrium and the branchial chamber thus become connected through the two gill-slits, while a similar union with the ectoderm on the dorsal middle line forms the atrial aperture.

Seeliger's account and figures show his persistency, and his account is as near to the truth as one could hope to get by the study of transverse sections of the stolon of Salpa democratica, but a very little study of sections in other planes, in more favorable species, will show that he has completely failed to understand the history, and that his account has little permanent value.

It is not only irreconcilable with my own observations, but also with our knowledge of pyrosoma, for both Seeliger (15, pp. 622–624) and Salensky (17, pp. 31–36) state that, in this genus, the perithoracic system is bilaterally symmetrical; that each bud has two perithoracic vesicles which are not dorsal and ventral, but right and left; that each of them unites with its corresponding half of the pharynx to form the gill-slits before the two vesicles unite with each other to form the median atrium, but that this arises, as it does in the aggregated salpa, on the dorsal middle line by the meeting and union of diverticula from the two vesicles, and that the external aperture arises still later, as it does in salpa, as an independent aperture on the middle line.

The perithoracic vesicles are derived, as they are in salpa, from the right and left perithoracic tubes of the stolon, but, in the primary ascidiozooids at least, these are continuous with the perithoracic tubes of the primary embryo or cyathozooid, where, according to both Kowalevsky (Arch. f. mik. Anat. XI, 1875) and Salensky (17, pp. 466, 473, 475), the evidence that they arise as paired ectodermal invaginations from the surface of the body is clear and unmistakable.

SECTION 10.—*The Digestive Organs of the Embryo.*

I have already, p. 31, described the way the cavity of the pharynx arises in the mass of visceral follicle cells by the degeneration of these cells. I have shown also that its endodermal epithelium is derived from the blastomeres, and, page 33, that the gut is formed as a diverticulum from the pharynx.

Salensky's account of the origin of the digestive organs is scattered through the pages of his memoir (5) in such a way that it is hard to review, and as I have already shown, page 293, that he has in the younger embryo mistaken the two perithoracic tubes for the pharynx, and that he has at a somewhat older stage mistaken the median atrium or cloaca for the pharynx, and that he has failed to discover the actual pharynx during its early stage, it is clear that his description has no value.

SECTION 11.—*The Endodermal Tube of the Stolon and the Digestive Tract of the Aggregated Salpa.*

On page 71 I have traced the origin of the endodermal tube of the stolon. My own observations on the origin of the pharynx by the union, on the middle line, of the two pharyngeal diverticula from the endodermal tube of the stolon have already been described, and as I have, page 95, outlined their complicated history to the best of my ability, there is little to add. I must repeat, however, that the pharynx is not actually, but only apparently, double in origin. While its oral and aboral ends consist, for a long time, of pouches which are separated from each other on the middle line, the middle section d' is never double. Fundamentally the pharynx is a single, unpaired expansion of the endodermal tube of the stolon, as it is in pyrosoma, and as I have represented it in the diagram on page 79; but at the time when it elongates towards the oral and aboral ends of the body, Plate V, Figs. 1 and 2, the middle line is occupied by the blood tubes, and it accordingly pushes along the sides of these structures, and does not become complete on the middle line until a much later stage.

The oral ends of the two pouches, Plate V, Fig. 4, unite very much sooner than the aboral ends, and in Salpa cylindrica these latter remain separated from each other, even after the body is fully formed, by a median ventral mesentery, Plate XXXIX, Fig. 11, which separates the right half of the pharynx, *27*, from the left half, *28*.

In Salpa pinnata the endostyle does not make its appearance until the halves of the pharynx are completely united with each other, and Plate XXXVI, Fig. 9, is the youngest stage in which I have found it in this species.   The persistency of the mesentery in Salpa cylindrica enables us to discover that the right half of the endostyle is formed in the right pharyngeal pouch, and the other half in the left.   In Plate XL, Figs. 14 and 15, the left half, *d 2*, of the endostyle of the right-hand salpa, and the right half, *d 1*, of the left-hand salpa, are shown diverging from the other halves and passing on to the far side of the stolon.

The post-pharyngeal gut arises as a blind diverticulum from the aboral end of the right pharyngeal pouch, *27*, as is shown at *q* in Plate V, Fig. 3, for a right-hand salpa, and at *q* in Fig. 4 for a left-hand salpa. The part of this diverticulum which joins the pharyngeal pouch becomes the œsophagus, and the stomach and intestine are developed from its blind end.   In all the species I have studied, the intestine bends to the left around the stomach to open directly into the median atrium, and the digestive tract assumes the form shown in Plate VIII, Fig. 2, and Plate XXXIX, as already described, page 187.   As the gut arises in both the right-hand and left-hand salpæ from the right-hand pharyngeal pouch, and since the distortions which are produced by pressure and by the changes of position affect the right-hand pouch of a right-hand salpa just as they do a left-hand pouch of a left-hand salpa, and as they affect the other pouch in quite a different way, the superficial history of the gut in a right-hand salpa is quite different from its superficial history in a left-hand one, although fundamentally they are exactly alike.

While Salensky, in his first paper on the budding of salpa (Morph. Jahrbuch, II, 1877), describes the endodermal tube, he says that it takes no part in the construction of the salpæ, and that their digestive organs are derived from that part of the stolon which I have called the genital rod.   Seeliger, a few years later, (11), p. 14, pointed out Salensky's error, which he has himself admitted in a recent paper, (17), p. 78.

Seeliger's account (11) of the origin of the endodermal tube and the digestive organs is given on pp. 14, 18, 26–34 and 54–62.   He shows, p. 14, that the endodermal tube of the stolon is derived from the pharynx of the embryo, with which it at first communicates.   He says that this connection is soon lost, but I have shown, p. 71, that it persists at all stages in the history of the stolon of Salpa pinnata and Salpa cylindrica.

He gives, p. 18, a good description of the segmentation of the side wall of the endodermal tube, but he says that the endoderm and mesoderm are the active agents in the segmentation of the stolon. He does not, however, figure any sections like those in my Plate XLIV, Figs. 5 and 11, Plate XXIII, Figs. 6–10, and Plate XXIX, Figs. 4–9; these sections show clearly that the active agent is not the mesoderm nor the endoderm, but, as I have stated on p. 78, the ectoderm.

He states correctly that the structures which I have called the pharyngeal pouches, arise from the side walls of the endodermal tube of the stolon, and that two of them enter into the body of each salpa; but here the agreement between his account and my own observations ends, although his figures show clearly that the species which he studied agree essentially with Salpa pinnata and Salpa cylindrica. While the two pouches are actually right and left, he regards one as dorsal and the other as ventral, and says that the dorsal one is the largest, and that it lies from the first on that side of the stolon to which the salpa belongs, and that it runs through the whole length of the body of the young chain-salpa, and opens, in its middle region, into the horizontal endodermal tube of the stolon, so that a neural and a hæmal part are distinguishable.

His account and figures show that his so-called dorsal pouch is actually the right-hand pouch of a right-hand salpa and the left-hand one of a left-hand salpa. In other respects his account of this pouch is correct, although he fails to discover that all the fundamental anatomical relations of the second pouch are exactly the same, as Plate V, Figs. 1 and 2 show.

He describes the second pouch as ventral and much smaller, and as lacking the oral end, and he says that it loses, long before the dorsal pouch, its connection with the horizontal tube, and becomes a closed vesicle; and a comparison of his figures with my own will show that he has failed completely in his interpretation of his sections, since all of the points which he notes are superficial and secondary.

He says that, as the hinder ends of the bodies of the salpæ diverge from each other, the small or ventral pouch pushes farther backwards than the larger dorsal one, and that the hindermost end of the dorsal pouch gives rise to a diverticulum, which grows round the hinder surface of the ovary to unite with the ventral pouch. The dorsal pouch gives rise to the pharynx, on the ventral surface of which the endostyle is developed, while the œsophagus, stomach, and intestine are formed from

the rudiment which has arisen at the posterior end by the union of two pouches.

It is not necessary to enter into a minute analysis of his description, for comparison of his figures with mine shows that he has been misled by his erroneous conception of the primary position of the buds and has mistaken the right and left pouches for dorsal and ventral.

His more recent account (15, pp. 23–25) of the origin of the post-pharyngeal gut of pyrosoma is very similar to what I have found in salpa, for he says (pp. 615–622) that, while it arises as a pair of folds from the pharynx, these soon unite to form an unpaired blind diverticulum, which afterwards becomes differentiated into œsophagus, stomach and intestine; that its pharyngeal end becomes the œsophagus, while the intestine arises from its blind end and ultimately acquires an anal opening into the median atrium.

Salensky's account of the origin of the gut in pyrosoma is quite different, however, for he says (17, pp. 69–72) that it is bilateral in origin and arises from a pair of folds from the sides of the aboral end of the pharynx, which unite with each other to form a horseshoe-shaped canal. If I understand his description, he holds that the right fold forms the œsophageal portion of the gut and the left one the intestinal portion, and that both open at first into the pharynx, although the intestine soon loses this connection and acquires a new anal opening into the median atrium.

Section 12.—*The Heart and the Eleoblast.*

The origin of the eleoblast of the solitary salpa is described on pp. 37–39, and the origin of its heart on pp. 41 and 42. The heart and the stoloblast of the aggregated salpa are formed from the mesoderm, which is described in Section 8, p. 76, and the origin of the heart is described on p. 83.

In all the aggregated salpæ which I have studied the stoloblast arises as a pair of symmetrical rudiments, as is shown at K on pp. 81 and 82, cuts O, P, Q, R and S. In Salpa pinnata these soon meet and fuse on the middle line, but in Salpa cylindrica, Plate XXXIX, Figs. 1 and 6, k, and in Salpa africana, they remain distinct even after all the other organs of the body are fully formed.

It is not recognizable as a distinct structure until the stage shown in Plate V, Figs. 2 and 3, is reached, but I see no reason for doubting its derivation from some of the mesoderm cells which are shown, uncolored, between the perithoracic tubes and adjacent tissues, in Plate XXIII, Fig. 7.

As the eleoblast of the solitary salpa is median and unpaired, the separation of its rudiment into halves in the aggregated salpa is, like the separation of the pharynx into paired pharyngeal pouches, a secondary adaptation to the conditions which are imposed by the peculiar method of budding. The morphological middle line of the body is occupied by the blood tubes of the stolon, and the rudiment of the stoloblast has thus been pushed to the right and to the left until it has become completely divided, during the ontogenetic history, into halves.

The point has theoretical interest. The stoloblast of the aggregated salpa is unquestionably homologous with the eleoblast of the solitary salpa, and is, therefore, a degenerated tail, as I have shown on page 38, but the eleoblast of the solitary salpa is median, and the cause of its separation into halves in the aggregated form is clear.

Davidoff has shown (16), p. 585, that since the middle line of the body of the distaplia embryo is occupied by the blastopore when the notochord and nervous system are first differentiated, these arise as paired organs. The history of the eleoblast of salpa shows that we cannot regard this as proof that the notochord and nervous system are phylogenetically paired, for the same reasoning would force us to regard the pharynx of salpa as, in origin, a paired organ.

SECTION 13.—*The Ovary and Testis.*

The origin and the homology of the ovary of salpa have been fully discussed in Chapters IX and X, and I have nothing more to add.

In my first paper on salpa (The Development of Salpa, Bull. Mus. Comp. Zool., 1876, No. 14) I advanced the opinion that the testis of the chain-salpa is homologous with the eleoblast of the solitary salpa, a view which is, of course, totally untenable at present. Salensky was almost equally unfortunate in the view which he advanced a year later (3), for he derives the genital rod of the stolon from the eleoblast of the solitary salpa, and holds that the testis of the chain-salpa is not derived from the genital rod, but that it arises, in place, from "mesoderm" cells.

Seeliger, nearly ten years later (11), p. 74, showed that the testis actually arises from the follicular epithelium of the genital rod, and my own observations show that this view is correct, although I have on p. 230 given my reasons for rejecting Seeliger's theoretical deductions from its history.

PART FOUR.

THE EYES AND SUBNEURAL GLAND OF SALPA.

By MAYNARD M. METCALF,

Fellow of Johns Hopkins University.

WITH PLATES XLVII TO LVII.

INTRODUCTION.

At Professor Brooks' suggestion I undertook a study of the eye of salpa in connection with his more extensive study of the genus. In the course of the work I discovered that salpa shows a more or less well-developed sub-neural gland, a point of so much interest that I include a description of this organ in the present paper. I am indebted to Prof. Brooks for the material upon which I worked and for the use of his own prepared sections of the embryos and developing stolons of Salpa pinnata. During the progress of this work I have received much assistance from him as my instructor, and I take this opportunity to express to him my warmest thanks.

The extensive collections of salpa made by the United States Fish Commission, which were placed at the disposal of Professor Brooks and upon which the investigations here recorded were made, include the following species :[1]

Cyclosalpa[2] pinnata, Forsk., solitary and chain forms.
Cyclosalpa Chamissonis, Brooks, chain form.
Salpa cylindrica, Cuv., solitary and chain forms.
Salpa runcinata-fusiformis, Cham., solitary and chain forms.
Salpa africana-maxima, Forsk., solitary and chain forms.
Salpa costata-Tillenii, Quoy and Gaim., solitary and chain forms.
Salpa hexagona, Quoy and Gaim., solitary and chain forms.
Salpa democratica-mucronata, Forsk., solitary and chain forms.
Salpa cordiformis-zonaria, Quoy and Gaim., solitary and chain forms.
Salpa scutigera-confederata, Cuv., solitary and chain forms.
Salpa bicaudata (?), Quoy and Gaim., chain form (probably to be regarded as a very distinct *variety* of Salpa scutigera-confederata).

METHODS.—Tunicate tissue is so easily manipulated that the simplest methods have given the best results. For staining, Kleinenberg's

[1] I have followed for the most part the nomenclature given by M. P. A. Traustedt [19]. (See list of papers referred to at the end of this memoir.)

[2] Whether the group cyclosalpa be regarded as a separate genus or as a sub-division of the genus salpa, it is convenient for the purposes of description in this paper to distinguish between the cyclosalpas and the salpas.

hæmatoxylin was used almost exclusively. For surface views the specimens were studied unstained, or mounted in a mixture of glycerine and acetic acid strongly tinged with methyl green.

Up to the present time no comparative study of the eyes of the different species of salpa has been made. Perhaps this is not strange, for the group salpidæ is so highly specialized that a knowledge of the eye of this group is not likely to throw much light upon the relation between the visual organs of the chordata in general. A very casual glance at the structure of the eye in the different species of salpa is enough, however, to show that such a comparative study is likely to prove of considerable value within the group.

So far as I can learn, the ciliated funnel is the only structure as yet described in salpa that has been regarded as homologous with any part of the sub-neural gland of ascidians.[1] In the course of the present paper I shall describe certain other structures, till now undescribed (see footnote), that seem beyond question to have the closest relation to the ascidian gland itself and to the lateral ducts from the gland of phallusia mammillata to the peribranchial chamber. I shall try to show also that a certain part of the ganglion of salpa is homologous with the sub-neural gland of ascidians rather than with any part of the ascidian ganglion.

SECTION 1.—*The Eyes.*

DESCRIPTIVE.

The most noticeable feature of the anatomy of the eye of salpa and cyclosalpa is its quite uniform shape throughout the solitary forms of the different species, and the strongly contrasted diversity of form that it shows in the chain individuals of these same species. These diverse forms are constant and characteristic for each species. In no case does the shape of the eye in the chain-form agree with the shape in the solitary form of the same or any other species. The variations in the histological structure must be described in detail. The eye of the chain individual is closely related to that of the solitary salpa, for it passes through an ontogenetic stage corresponding in shape to the adult condition of the latter. The eye of the solitary salpa must then be regarded as the type from which the eye of the chain-salpa has diverged to a

[1] In a preliminary notice of this paper I gave a brief description of the organs here described at greater length [12].

greater or less extent in the different species. The eye of the chain-salpa is not, however, in all cases a simple structure with a single origin, as is the case in the solitary form, but in several species distinct eyes are developed, or new portions of distinct origin are added to that part of the eye which is homologous with the eye of the solitary form.

First I will describe the adult eye of the solitary Cyclosalpa pinnata and its development, as the type to which to refer. Then I will describe the adult eyes of the chain form of the same species and trace their development. With this as a foundation we will pass to the study of the other species.

THE ANATOMY OF THE EYE OF CYCLOSALPA PINNATA, SOLITARY FORM.

On the dorsal surface of the ganglion of the solitary form of Cyclosalpa pinnata there is a ridge shaped like a horse-shoe, with the open end of the horse-shoe anterior. (Compare Fig. 7, Plate LVI, which represents the eye, ganglion, and adjacent parts of the solitary Salpa scutigera-confederata. The condition in the solitary Cyclosalpa pinnata is essentially the same.) This ridge, like the ganglion, is composed of a cellular peripheral portion and a non-cellular core; the cellular portion of the one being continuous with that of the other, while the core of the ridge is continuous with the central, non-cellular portion of the ganglion (Plate LI, Fig. 8). The height of the ridge above the ganglion is a little greater than its width. On the anterior face of the posterior part of the ridge and on the inner faces of its two anterior limbs, the cells are modified to form the retina of the eye (Plate LI, Fig. 8). The eye, then, in every part faces toward the mid-dorsal point of the brain. The retina is formed from the more dorsal cells of the inner half of the optic ridge (that half toward the mid-dorsal point of the brain). The cells of the outer half of the ridge and of the ventral part of the inner half exactly resemble the cells of the ganglion. In Cyclosalpa pinnata, these cells, which are not shown in the figure of the eye of Salpa scutigera-confederata, would appear in surface view as a thin layer outside the pigment.

As seen in Plate LI, Fig. 8, the ectoderm lies on the outer and dorsal surfaces of the optic ridge, but does not descend to the ganglion between the anterior limbs of the ridge. Between the dorsal surface of the ganglion, the optic ridge and the ectoderm there is a space (oc) which is a blood lacuna. This space, the optic chamber, is nearly shut off from the space in which the ganglion lies by a membrane (z') which stretches from the ganglion, at the base of the optic ridge, out on all sides to the

ectoderm. On the mid-line in front of the ganglion there is a perforation of this membrane, through which the circumganglionic blood space freely communicates with the optic chamber. The delicate membrane that clothes the ganglion is continued over the optic ridge. The membrane that closes off the optic chamber from the space in which the brain lies is continuous, on the one hand, with the membrane that covers the ganglion and eye, and, on the other hand, with the basement membrane upon which the ectoderm cells abut. That portion of the ectoderm which covers the eye and bounds the optic chamber I propose to call the optic sheath (Plate LI, Fig. 8, *os*).

The histological structure of the retina is the same in all regions. Fig. 8, Plate LI shows a cross-section of the ganglion and of the two anterior limbs of the optic ridge. Figure 9 is a more enlarged drawing of the retinal portion of the left limb shown in Fig. 8. The retina consists of three kinds of cells: (1), rod cells; (2), intermediate cells; (3), pigment cells. The rod cells (Fig. 9, *r* and *s*) are elongated columnar; their long axes being parallel to the horizontal plane of the body of the salpa. They each consist of two portions: *s*, a thin-walled, lightly staining, outer portion (away from the core of the ridge) containing very finely granular protoplasm and a large nucleus with nucleolus and very apparent chromatin network; and *r*, a thick-walled, deeply staining, inner portion abutting on the next inner layer (2). The deep stain of the inner third of the rod cells is due to the thickness of the cell walls, which take the stain much more readily than the protoplasm does.

In the second layer (2), (Fig. 9, Plate LI, *i*) no cell boundaries can be made out, but the presence of a number of nuclei exactly resembling the nuclei of the peripheral portion of the ganglion indicates the cells of the second layer, for which I propose the name *intermediate cells of the retina*. The cell boundaries in the peripheral portion of the ganglion are no more visible than in the intermediate layer of the retina. These intermediate cells are ganglion cells which have remained unaltered during the development of the eye from the cells of the brain. In each section the number of intermediate cells is about equal to the number of rod cells. I have been unable to study live salpas, so could not make successful macerations. I am convinced, however, that the intermediate layer of the retina is not a multinucleated mass of protoplasm, but is composed of true cells. In a partially successful maceration of the peripheral layer of the hardened ganglion, whose cells exactly resemble in appearance the intermediate cells of the retina, I could make out that the ganglion cells were of irregular shape and had, in some cases, as many

as three processes.   In Salpa runcinata-fusiformis, chain form, the cells of the intermediate layer are distinct and show usually a single process toward the rod cells.

The third layer of the retina (3) is the pigment layer (Fig. 9, *p*), composed of cells so full of pigment granules and so closely massed together that no structure can be made out.   A study of their development and a comparison with other species show them to resemble, save for the pigment they contain, the cells of the intermediate layer of the retina or the ordinary cells of the ganglion.   These pigment cells are arranged in a semicircle enclosing the intermediate cells and the inner ends of the rod cells.

I did not find the innervation of the eye in the solitary Cyclosalpa pinnata, but in the solitary Salpa democratica-mucronata (Plate LV, Fig. 10), the outer, thin-walled ends of the rod cells receive fine fibers that come from the dorsal part of the ganglion, apparently pushing out from the non-cellular core, through the peripheral cellular portion.   It is probable that the eye receives fibers also directly from the dorsal cells of the ganglion.   The distinctive histological character of the retina is seen from this description to consist of but two kinds of modification of the ordinary ganglion cells: first, the pigmentation of certain cells otherwise unmodified; second, the more complicated differentiation of the rod cells. No lens is ever present.   The eye must be a very efficient light-perceiving organ, but the arrangement of the rod cells, the total absence of any lens and the simple character of the whole organ seem, beyond doubt, to show that it can give no perceptual image.

THE DEVELOPMENT OF THE EYE OF CYCLOSALPA PINNATA, SOLITARY FORM.

At a time in the development of the embryo, when the central cavity of the nervous system has just been obliterated, and the central cells of the ganglion are degenerating to form the central, non-cellular core, the cells destined to form the optic ridge push up from the dorsal surface of the ganglion.   These cells are arranged, from the first, in a ridge having the characteristic horse-shoe shape, the open end of the horse-shoe being anterior.   Salensky[1] describes the embryonic solitary

[1] " Im Nervenganglion kann man nun zwei Theile unterscheiden : einen unteren— das eigentliche Ganglion—und einen oberen—die Anlage der Augen.   Letztere setzt sich vom ersteren durch eine Einschnürung ab und verlängert sich nach vorn und hinten in zwei solide Fortsätze—die Anlage der Augen.   Die Augen sind durch eine mittlere Brücke mit einander verbunden."   W. Salensky [15].

cyclosalpa eye as double; the two halves, right and left, being connected by a posterior transverse band. This description is a little misleading, for the size of the posterior curved part of the retina and optic ridge is fully as great as, and in most species a little greater than that of either of the two anterior limbs, and it is an equally important part of the eye. The eye forms a continuous curve a little greater than a semicircle, and is a single structure in its adult form and in its origin. In the chain form of different species the type is departed from and the eye is in two cases strongly bilobed, as will be shown further on.

The ridge increases in size as the ganglion grows. As the central cells of the ganglion degenerate, the cells of the core of the ridge also degenerate; the two areas not being separate, but being, from the first, continuous with each other. Fig. 5, Plate LI, represents a section of the ganglion and eye at a time when the degeneration described has made some progress. It is a vertical transverse section through the central part of the ganglion. On the dorsal surface are seen sections of the two anterior limbs of the developing optic ridge. Fig. 6 shows a more posterior section of the same series, cutting the posterior curved portion of the horseshoe-shaped ridge.

At a considerably later period the retina cells begin to assume their characteristic appearance. The first noticeable change is the enlargement of the most peripheral layer of cells over the dorsal portion of the ridge. They elongate and become columnar, with their long axes dorsoventral (Plate LI, Fig. 7). Ventral to the rod cells there are about three times as many cells having the character of the ordinary ganglion cells. About one-third of these will remain unmodified to form the intermediate cells of the retina. At about this time the other two-thirds begin to become pigmented, and, a little later, the walls of the inner third of each rod cell become thickened and so stain more deeply. The adult condition is reached by an increase in the size of the rod cells, by a greater thickening of the walls of their inner ends, by a greater deposit of pigment in the inner cells of the retina, and by a shifting of the retinal area from the dorsal surface to the dorsal part of the inner surface of the ridge. (Compare Fig. 7 with Fig. 8, Plate LI.) This change of position seems to be caused by the greater growth of the outer face of the optic ridge, pushing dorsalward that edge of the retina which was most distant from the mid-dorsal point of the brain (p in Fig. 7, Plate LI). Of course, by this shifting the long axes of the rod cells, which were originally vertical (Fig. 7), become horizontal (Fig. 8).

THE ANATOMY OF THE EYES OF CYCLOSALPA PINNATA, CHAIN FORM.

The histological structure of the retina of the dorsal eye of the chain form of Cyclosalpa pinnata agrees closely with that of the solitary form, save that no intermediate layer of cells is present between the rods and the pigment cells, *i. e.*, all the cells of the retina except the rod cells have become pigmented. The shape of the eye, on the other hand, differs greatly from that found in the solitary form. There are present in the chain Cyclosalpa pinnata two pairs of small eyes, till now undescribed,[1] in addition to the well-known unpaired dorsal eye.

The Unpaired Dorsal Eye.

This is situated on the dorsal surface of the ganglion (Plate LVII, Figs. 9 and 10) in a position similar to that occupied by the eye of the solitary form, except that it extends beyond the anterior face of the ganglion, only the posterior third of the eye lying upon the brain (Plate LVII, Fig. 10, and Plate XLIX, Figs. 1 and 2). It consists of two almost distinct portions, the larger of which may be described as a horseshoe with the open side of the horseshoe posterior, differing in this respect from the eye of the solitary form, which has its open side anterior. (Compare Plate LVI, Fig. 7, with Plate LVII, Fig. 9.) The posterior ends of the two posterior limbs are enlarged, as shown in the figure, so that the description "horseshoe-shaped" is not strictly applicable to this portion of the eye. The second smaller portion (*e'''*) lies in the curve of the anterior part of this horseshoe. It is elongated transversely, reaching from one limb of the horseshoe to the other. It is connected to the rest of the eye only by a number of spindle-shaped cells, binding the anterior face of the second mass to the posterior face of the anterior portion of the main body of the eye (Plate LVII, Figs. 9 and 10, and Plate XLIX, Figs. 1 and 2). In any one longitudinal section of the eye four or five of these spindle cells appear. This eye, like the eye of the solitary form, lies immediately beneath the ectoderm, in a chamber wholly shut off from the space in which the ganglion lies, except for a narrow opening on the mid-line in front. The optic chamber is, then, a blood space connected with the blood sinus in which the brain lies. In Plate XLIX, Figs. 1 and 2, which are longi-

---

[1] In my preliminary note of this paper [12] I described briefly the structure, position and development of these smaller eyes.

tudinal sections, and Figs. 4 and 5, which are cross-sections, the shape of this cavity is shown. The membrane, which intervenes between the optic chamber and the space in which the ganglion lies, is shown in all four figures at z'. The rupture of this membrane in Fig. 2 is the point of connection between these two blood spaces. The membrane is seen to be continuous, on the one hand, with the delicate membrane around the ganglion and, on the other hand, with the membrane upon which the cells of the ectoderm rest. Figs. 1 and 2, Plate XLIX, show the characteristic infolding of the ectoderm between the anterior part of the eye and the dorsal surface of the ganglion. The only parts of the eye that abut on the ganglion are the posterior ends of its two posterior limbs. All the rest is separated from it by a double fold of ectoderm. In Fig. 5, Plate XLIX, which is a vertical cross-section of the ganglion and eye, through the region designated by e' in Fig. 1, there is shown the double fold of ectoderm, k, separating the eye, e', from the ganglion. The delicate membrane that clothes the ganglion is continued over the whole eye (Fig. 1).

The arrangement of the histological elements is different in different regions (see Plate XLIX, Figs. 1 and 2). In the posterior limbs, e', the rod cells are dorsal and the pigment, p', ventral. In the anterior curved part of the eye, e'', this arrangement is nearly reversed; the pigment, p'', being on the dorsal and posterior faces, while the rods are on the ventral and anterior faces. Where these two regions, e' and e'', meet at the antero-lateral angles of the eye, the pigment layer of the one bends toward, but is not quite continuous with that of the other; the two almost meeting on the inner face of the eye, $i.\ e.$, on that face which looks toward the mid-dorsal point of the ganglion. There is, then, a twisting of the main body of the eye, causing the pigment layer, as we pass from behind forwards, to face first ventrally, then toward the middle line, and finally dorsally and posteriorly. On the other hand, the same twisting causes the rod cells to face first dorsally, then laterally, and at last anteriorly and ventrally.[1] In the second, smaller portion of the eye, which lies in the anterior curve of the main portion, the rod cells face posteriorly toward the open end of the horseshoe, while the pigment layer faces anteriorly, lying close to the pigment layer of the anterior part of the main body of the dorsal eye.

[1] I do not mean that there has been an actual twisting, but that there is seen in the eye a spiral arrangement of its elements such as would result from twisting. The development (see below) shows there has been no actual twisting.

The eye is innervated by two optic nerves that arise in the non-cellular core of the ganglion (Plate XLIX, Figs. 1, 4 and 6, *on*). The fibers run in two bundles, one on each side, over the posterior and dorsal faces of the two posterior limbs of the eye, some of them here entering the clear ends of the rod cells. Further forward, at the point where the relative position of rod cells and pigment cells is reversed, the fibers on each side divide into two bundles; one bundle going directly to the clear ends of the rod cells of the second, smaller portion of the eye (Fig. 2); the other passing around the inner side of the eye, below the secondary portion, to innervate in the same way the rod cells of the anterior portion of the eye (Plate XLVIII, Fig. 9, *on″*). The origin of the two optic nerves is shown in Fig. 4, Plate XLIX, which represents a cross-section of the ganglion, through the region designated by *on* in Fig. 1. Fig. 6 is an enlarged drawing of the section of the left limb of the eye shown in Fig. 5. In this figure the optic nerve, *on*, appears on the dorsal surface; ventral to this are the rod cells with their clear ends, *s*, containing large nuclei, and their thick-walled, deeply staining ends, *r*; ventral to these and abutting directly upon them is the layer of crowded pigment cells, *p*, so full of pigment granules that no structure can be made out. Over the whole surface of the retina the delicate membrane, *z*, is seen.

In one case the dorsal eye was removed from a hardened specimen of the chain Cyclosalpa pinnata and, after maceration in Haller's fluid, was gently torn with teasing needles. A bit of the smaller portion of the eye, *e‴*, was in this way isolated with some of its nerve fibers still attached. Fig. 7, Plate XLIX, which represents an optical section of this piece, shows very clearly the separate fibers connecting with single rod cells. This was even more clearly shown in more finely teased portions. The innervation of the eye is well shown in Figs. 7 to 9, Plate XLVIII, which represent sections of the nearly mature eye of one of the older individuals attached to the stolon of the solitary salpa.

In Fig. 3, Plate XLIX, is seen a section of a portion of this eye cutting the thick-walled ends of the rod cells at right angles to their long axis. The walls of adjacent cells are so closely pressed together that they seem to form a single continuous network. In my unsuccessful macerations of the hardened eyes I could see in several cases a slight separation of the rods of adjacent cells, but the preparations were not sufficiently satisfactory to draw. (See Fig. 7, Plate XLIX, which shows imperfectly the same thing.) The appearance indicated that this seeming network is not a continuous structure, but rather

that each cell with its thickened walls is a separate structure, and that the deceptive appearance of a network is caused by the close apposition of the thick walls of adjacent cells. This is, of course, what we would expect reasoning from analogy with the rod cells of other eyes.

In the same figures is noticed the protoplasmic core of each rod cell, which penetrates clear to the extremity of the thick-walled end of the cell. Within these protoplasmic cores are seen here and there (Fig. 3) round, or slightly oval, homogeneous, deeply staining bodies somewhat resembling nuclei.[1] These are found only in the thick-walled ends of the cells, and each cell contains one of them. They seem not to be true nuclei, but to correspond to the very similar bodies found in the rod cells of the eyes of other animals, e. g., the pycnogonids (Morgan), insects ("secondary nuclei," Patten), scorpions (Lankester and Bourne), and probably also to the refractive globules in the cones of the retina of birds.

The Two Pairs of Smaller Eyes.

The four smaller eyes of the chain-form of Cyclosalpa pinnata are arranged in two pairs, one pair lying on the posterior face of the ganglion on each side of the middle line, the other pair lying just below the posterior ends of the two posterior limbs of the unpaired dorsal eye. Fig. 8, Plate XLIX, which represents part of a longitudinal, vertical section of the ganglion, shows one of each pair of smaller eyes, ex and ey. Fig. 1, Plate LI, a vertical cross-section of the ganglion, shows the two eyes of the posterior pair. They lie imbedded in the midst of the smaller cells of the ganglion, just dorsal to the zone of origin of the nerves that arise from the brain. These eyes consist simply of rod cells which exactly resemble, except in size, the rod cells of the large dorsal eye. These cells are arranged in a hemisphere with their thin-walled, lightly staining ends posterior and their thick-walled, deeply staining ends toward the center of the ganglion. The membrane of the posterior face of the ganglion touches the posterior ends of the rod cells. In the specimens studied, all of which were hardened in acid reagents, no pigment was found in the region of these eyes. In the live cyclosalpa it may be that certain of the ganglion cells near the base of the rods are lightly pigmented; but this pigmentation cannot be very decided, for if ever present in the specimens studied, it had been dissolved by the same

[1] In the figure they are represented as granular. They should be homogeneous, staining a little less deeply than the cell walls.

reagents that had left apparently unimpaired the pigment granules in the pigment cells of the large dorsal eye.

The structure of the dorsal pair of small eyes is the same as that of the posterior pair. In this case, however, the rod cells, which are arranged in a hemisphere, have their nucleated ends pointing toward the center of the ganglion and their thick-walled ends abutting on the pigment layer of the large dorsal eye (Plate XLIX, Fig. 8). A comparison of Fig. 8, Plate XLIX, with Figs. 2 and 3, Plate LI, which are vertical transverse sections (a little oblique), will show the exact position of these eyes. The right one lies at the base of the right optic nerve, on the right side of and a little in front of it. The left one is situated in a corresponding position on the left side. The pigment layer of the unpaired dorsal eye is separated from the rod cells of each small dorsal eye by the delicate neural membrane that intervenes (Plate XLIX, Fig. 8). This may or may not prevent the small dorsal eyes functioning as light-perceiving organs in connection with the pigment of the large dorsal eye. It indicates that the small dorsal eyes cannot be regarded as parts of the larger unpaired eye.

THE DEVELOPMENT OF THE EYES OF CYCLOSALPA PINNATA, CHAIN FORM.

Dorsal Unpaired Eye.

The early stages of development of this eye in the chain form are almost identical with those in the solitary form. See Figs. 1 to 3, Plate XLVII, which represent a series of vertical transverse sections through the ganglion at the time of the earliest appearance of the rudiment of the eye. Fig. 1 portrays the more anterior section; Fig. 3 the more posterior. The rudimentary optic ridge is designated by e. It first appears at a time when the central cells of the ganglion are commencing to degenerate. It has, from the first, the characteristic horseshoe shape seen in the eye of the solitary cyclosalpa. The first two sections cut the two anterior limbs of the ridge. (Compare with Fig. 5, Plate LI, which represents an anterior section of the developing eye of the solitary form.) The third section cuts through the posterior curved portion of the horseshoe. (Compare with Fig. 6, Plate LI, a section through the posterior part of the developing eye of the solitary form.) The horseshoe-shaped arrangement of the cells of the rudiment of the eye is very soon lost, but it is always present for a short time. I have examined serial sections of the ganglia of more than one hundred individuals of about the age repre-

sented in Figs. 1 to 3, Plate XLVII, and have always found the same appearance of a definite, though slightly developed, horseshoe-shaped ridge. This appearance is so constant and uniform that we can safely say the eye of the chain cyclosalpa passes through a stage when it corresponds in shape to the eye of both the adult and embryonic solitary Cyclosalpa pinnata. The central cells of the ganglion and the cells of the core of the ridge degenerate at the same time, as is also the case in the solitary cyclosalpa.

Very soon the horseshoe-shaped arrangement of the eye cells is lost. They are, from the first, close pressed to the ectoderm. Soon after the appearance of the optic ridge the ectoderm arches up over the ganglion, carrying with it the cells of the ridge. The commencement of this process is seen in Fig. 5, Plate XLVII. The eye cells lose their connection with the ganglion, except that the non-cellular core of the ridge is pulled out into long fibers that bind the eye cells to the ganglion. The rudiment of the eye is now a thickened disk of cells, close pressed to the ectoderm, with fibers connecting the center of its ventral face with the non-cellular core of the ganglion. As development proceeds the anterior edge of the disk approaches the brain till it comes in contact with it (Plate XLVII, Fig. 7). While the anterior edge shifts its position, the posterior edge retains its former place, the disk becoming in this way nearly perpendicular to the dorsal face of the ganglion (Plate XLVII, Fig. 7, and Plate XLVIII, Fig. 1). The nerve fibers which, from the first, connected the center of the disk with the non-cellular core of the brain, now, of course, lie along the posterior face of the perpendicular disk (Plate XLVII, Fig. 7, and Plate XLVIII, Fig. 1, *on*). During its change of position the disk remains in connection with the ectoderm, causing the latter to approach anteriorly almost to the surface of the ganglion. The posterior edge of the disk, during this shifting of position, curls over backwards, forming the first rudiment of the second mass of retinal tissue which lies in the anterior curve of the main body of the adult eye (*e'''* in Fig. 7, Plate XLVII, and Fig. 1, Plate XLVIII). The whole eye continues to bend forward till it comes to lie horizontal with the originally anterior edge of the disk posterior and the originally posterior edge anterior. (Compare Figs. 1, 5 and 7, Plate XLVIII.)

The histological differentiation of the retinal elements, the change of form of the eye and its shifting from a perpendicular to a horizontal position, proceed simultaneously, all three processes becoming complete at the time when the circle of chain cyclosalpas is set free from the mother-

stolon. (Compare Fig. 7, Plate XLVII, and Figs. 1 to 10, Plate XLVIII.) As in the solitary Cyclosalpa pinnata, the rod cells are the first to distinguish themselves, appearing at the same time in all regions of the eye. Their cell boundaries become distinct. Soon they elongate, becoming columnar (Plate XLVIII, Fig. 1). After a very short interval the deeper staining of their inner ends shows the cell wall of this portion to be somewhat thickened (Fig. 5, Plate XLVIII, r). The adult histological condition is reached by the greater elongation of the rod cells, an increase in the size of their nuclei, a greater thickening of the cell walls of their inner ends, and by a very dense deposit of pigment granules in the cells of the pigment layer of the retina. This pigmentation does not show in any of the young cyclosalpas still attached to the stolon up to the time of the formation of the terminal wheel about to be set free. It must then be deposited rapidly between the time when this wheel is formed and the time when it is set free from the stolon of the solitary cyclosalpa.

During the change in the histological character and in the position of the eye there is a concomitant change of form. The change from the very early horseshoe-shaped ridge on the dorsal surface of the brain to the thickened disk close pressed to the ectoderm and connected with the brain only by nerve fibers, has already been noted. We have seen also that the originally posterior edge of this disk curls over backwards to form the rudiment of the second portion of the eye, seen in the anterior end of the main body of the adult eye (e''' in Fig. 7, Plate XLVII, and Figs. 1, 3, 5 and 6, Plate XLVIII). For a long time this rudiment is connected to the main body of the eye by a considerable mass of cells resembling the ordinary cells of the ganglion, $i.\ e.$, by eye cells that still retain their primitive character (Plate XLVIII, Fig. 9). When the wheel of chain cyclosalpæ is about to loose from the stolon this mass of cells differentiates into three portions, one forming the pigment layer of the anterior portion of the main body of the eye (Plate XLVIII, Fig. 8, p''), another forming the pigment layer of the secondary part of the eye; the cells of the third portion becoming elongated to form the spindle cells that bind the secondary part of the eye to the main body (Plate XLIX, Fig. 1, q). While the differentiation of this mass of cells in the three directions mentioned is taking place, the posterior end of the now horizontal eye is dividing, longitudinally, in a vertical plane to form the two posterior limbs seen in the adult eye. In Fig. 2, Plate XLVIII (which is a horizontal section of that portion of the eye represented by e' in Fig. 1, Plate XLVIII, at a corresponding stage of development to

that portrayed in Fig. 1), no indication of a division into two limbs is visible. Even in a much later stage (Fig. 6, Plate XLVIII, a vertical cross-section of the eye when it is perpendicular to the brain) no trace of such a division is seen. Later, when the young wheel is formed, but is still attached to the stolon, the division is complete (Plate XLVIII, Fig. 10), though the two limbs of the eye are not separated by so great a space as in the adult.

While the developing eye is shifting from a perpendicular to a horizontal position it remains attached to the ectoderm. The ectoderm is thus folded back upon itself, forming the double fold seen in the adult between the eye and the ganglion. (Compare Plate XLVII, Figs. 5 and 7; Plate XLVIII, Figs. 1, 5 and 9, and Plate XLIX, Fig. 1.)

The Small Paired Eyes.

The two pairs of small eyes are formed at a very late period. No trace of them is found in the chain cyclosalpa still attached to the stolon. Although their development has not been observed, they undoubtedly develop from the small ganglion cells, in the position they occupy when fully formed. Since in them no pigment layer or intermediate layer is distinguishable, the only change in the ganglion cells necessary to produce them would be a modification of certain of these cells into rod cells, after the manner of the development of the rod cells in the large eye of the chain or solitary form.

THE ANATOMY OF THE EYES IN OTHER SPECIES OF SALPIDÆ.

Cyclosalpa Chamissonis.

The eyes of Cyclosalpa Chamissonis are more closely related to those of Cyclosalpa pinnata than are the eyes of any of the true salpas. In the solitary form the eyes of the two are practically identical. In the chain form the dorsal eye corresponds to the immature dorsal eye of Cyclosalpa pinnata just before the latter is set free from the stolon (Plate LVII, Fig. 8). It has the same position, projecting beyond the anterior face of the ganglion, with only its posterior third lying on the dorsal surface of the ganglion. It is noteworthy that in this species the eye has not bent quite so far forward as in the adult Cyclosalpa pinnata, but is in a position corresponding to that seen in the eye of the imma-

ture Cyclosalpa pinnata (Fig. 9, Plate XLVIII). There are seen two portions of the eye, corresponding to the larger and smaller portions of the eye of Cyclosalpa pinnata. The two parts, however, are not so distinct as in that species. Instead of being bound together by a few spindle-shaped cells connecting the pigment layer of the one with that of the other, they are connected by a considerable mass of cells, all of which are pigmented and serve as the pigment layer for both portions of the eye. A comparison of Fig. 2, Plate LIII, with Figs. 6 and 9, Plate XLVIII, will show that the adult Cyclosalpa chamissonis has, in this respect, retained the condition found in the immature Cyclosalpa pinnata. A comparison of Fig. 1, Plate LIII, with Fig. 2, Plate XLVIII, and Fig. 5, Plate XLIX, will show, also, that there are in Cyclosalpa chamissonis no distinct posterior limbs of the eye, but that this portion is an undivided, continuous structure, retaining the condition found in the eye of the immature chain Cyclosalpa pinnata. The relative arrangement of the histological elements in the three regions of the eye (*e'*, *e''*, *e'''*), and also the histological structure and the innervation, are the same in both species; except that the rod cells are shorter in the posterior part of the eye of Cyclosalpa chamissonis. The *histological structure* of the dorsal eye of the chain form of the latter species corresponds, then, to the condition found in the *adult* Cyclosalpa pinnata, while the *position* and *form* of the eye correspond to that found in the *immature* Cyclosalpa pinnata. *The unpaired dorsal eye of Cyclosalpa pinnata passes through a chamissonis stage.* The marked similarity in form, structure and position of this eye in the two species makes it certain that, in the course of its development, the dorsal eye of the chain Cyclosalpa chamissonis undergoes a shifting of position similar to that described for Cyclosalpa pinnata, so that in each of these species the anterior end of the eye was primitively posterior, and *vice versa*.

The two pairs of smaller eyes found in the chain form of Cyclosalpa pinnata are represented in the chain form of Cyclosalpa chamissonis by two pairs of small eyes with the same form and structure and nearly the same position as in Cyclosalpa pinnata; the posterior pair being a little more dorsal than in the latter species.

The structure of the eye in these two species confirms the conclusion drawn from other anatomical resemblances, that the two are closely related, Cyclosalpa chamissonis being the more primitive, and that they are somewhat removed from the other salpidæ.

SALPA CYLINDRICA.

The eye of Salpa cylindrica, in both the solitary and chain forms, presents more marked differences from the conditions found in Cyclosalpa pinnata. In the *solitary form* the only point of difference is that the rod cells of the posterior curved part of the horseshoe are directed ventralward instead of forward, and the pigment layer is dorsal (Fig. 1, Plate LII). In the two anterior limbs of the eye the condition is the same as in the corresponding regions in Cyclosalpa pinnata (Fig. 2, Plate LII).

In the eyes of the *chain form* there are very marked features of difference from Cyclosalpa pinnata. Fig. 4, Plate LVI, gives a surface view, and Fig. 4, Plate LII, shows the general structure. The larger eye is on the dorsal surface of the ganglion, projecting a little, but not far beyond its anterior face. There is a single large optic nerve entering the posterior part of the eye. The latter figure shows that there are two eyes: one larger (e', e''), plainly seen in surface view (compare Fig. 4, Plate LVI); the other, smaller (ex), placed behind the former on the dorsal surface of the ganglion (Fig. 4, Plate LII). A vertical cross-section of the ganglion, through this smaller eye, is shown in Fig. 8, Plate LII, and a more enlarged drawing is given in Fig. 9 of the same plate. The appearance shown in longitudinal section is the same (Fig. 4, Plate LII, ex), indicating that the organ is circular. This eye corresponds, I believe, to the dorsal pair of smaller eyes seen in Cyclosalpa pinnata. It agrees with the latter in histological structure and in position, only that the two have fused together in Salpa cylindrica across the middle line behind the optic nerve. Notice that the thin-walled, nucleated ends of the rod cells of the small eye point toward the core of the brain, as do the rod cells of the small dorsal eyes of Cyclosalpa pinnata. The fusion of the two small dorsal eyes into one in Salpa cylindrica corresponds to the more compact form of the larger eye and the fusion of the two optic nerves into the single nerve found in the latter species. For convenience of description I refer the structure of the eyes in the different species to that found in Cyclosalpa pinnata. I believe, however, that the eye of that species is not primitive, but is the most removed from the ancestral condition. We start, then, in our study with the more modified forms and pass to the less modified. Fig. 9, Plate LII, shows the innervation of the smaller eye. The fibers that come to it arise from the non-cellular core of the brain, in close connection with the optic

nerve. There is no organ in Salpa cylindrica corresponding to the posterior pair of small eyes in Cyclosalpa pinnata.

The structure of the larger eye, which, I believe, corresponds to the large, unpaired dorsal eye of Cyclosalpa pinnata, is shown in Figs. 5 to 7, Plate LII, which represent vertical cross-sections through three regions of the eye. Fig. 5 represents the more posterior section cutting the eye in front of the origin of the optic nerve (compare Fig. 4, Plate LII). The optic nerve is shown on the dorsal side at on; the rod cells at s'; the pigment layer at p'. The layer of intermediate cells is mostly wanting, though a few do show here and there. In other words, the posterior cells of the retina have almost all become pigmented. In this region the rod cells are seen to be dorsal and the pigment layer ventral. In the anterior region of the eye this arrangement is reversed, the rod cells being ventral and anterior and the pigment dorsal (Figs. 6 and 7, Plate LII). There is no secondary portion of the eye corresponding to that which lies in the anterior curved portion of the large dorsal eye of Cyclosalpa pinnata, chain form. There are, however, two peculiar small lobes of the eye (a in Figs. 5 and 6, Plate LII). The long axis of the eye is not parallel to the midline of the body, but is quite oblique (Fig. 4, Plate LVI), so that these sections, which are transverse to the long axis of the body, cut the eye obliquely. If it were not for this obliquity the two small lobes would appear in the same section in the region where the relative arrangement of the rod cells and pigment cells of the basal portion is reversed to give the condition found nearer the apex. They are a structure characteristic of this species, not being developed in any other. They cannot, without further study of the development, be homologized with the smaller portion of the dorsal eye of the chain Cyclosalpa pinnata, because of the exactly opposite relative position of the histological elements in the two cases.

SALPA RUNCINATA-FUSIFORMIS.

Solitary Form.

In the eye of Salpa runcinata-fusiformis we find quite marked variations from any of the forms described. In the solitary form we have the typical horseshoe-shaped retina in the usual position, but the development of retinal tissue at the anterior ends of the two anterior limbs is very great. In this region all the cells of the whole optic ridge are modified to form the retina. Figs. 4, 5 and 6, Plate LIII, represent cross-

sections of three regions of the eye of the solitary Salpa runcinata-fusiformis. The more posterior (Fig. 4) cuts the optic ridge through the posterior curved portion just at the origin of the anterior limbs. We see the core of the ridge at *cr* and the retina above it, *e*. In this species, as in Salpa cylindrica, the pigment layer of this portion of the eye is dorsal, *p*; next to this comes the layer of intermediate cells, *i*; ventral to this again are the rod cells. These are not definitely columnar and arranged in a single layer, but they are irregular in shape, have irregularly thickened walls and are arranged in an agglomerate mass. The rod cells are elongated in a dorso-ventral direction, suggesting vaguely the columnar structure of the corresponding cells of the retina of Salpa cylindrica or Cyclosalpa pinnata. Further forward, in the anterior limbs of the eye, the relative position of pigment cells and rod cells is changed, the former being on the outer, the latter on the inner face of the optic ridge (Fig. 6, Plate LIII). This corresponds to the position in other species. Fig. 3, Plate LIII, a longitudinal section through one of the limbs of the eye, shows the great development of retinal tissue, *eq*, at the anterior end of the eye. Fig. 6, Plate LIII, a cross-section of this region, gives a better conception. The anterior limbs are seen on the dorsal side, *e*; between the anterior limbs of the eye there is a great mass of rod cells, *eq*. They are best called rod cells because of their undoubted relation to the typically developed rod cells of other species, though in this species they do not have the ordinary structure of rod cells. This ventral extension of the anterior ends of the two anterior limbs of the eye is a peculiar feature not represented in the solitary form of any other species studied. The condition of the rod cells seems to indicate that the retina is degenerate, but rod cells of a very similar character are found in several species yet to be described, and it will be well to defer any discussion of the point till we can gain a comprehensive view of all such species.

Between the ectoderm and tunic, in the region above the ganglion and eye, there is a large cavity which in life would be full of sea-water. This would act as a cushion, and must be a very efficient protection for the ganglion and eye. Fig. 3, Plate LIII, shows the relation of this cavity, *ts*, to the adjacent organs.

Chain Form.

The eye of the chain form of Salpa runcinata-fusiformis (Plate LVI, Fig. 6) shows certain very interesting characters. In shape it is elon-

gated ovoid, with the more pointed end anterior. Its long axis is nearly horizontal, and is directed forward and about twenty degrees to the right or left. It lies wholly in front of the ganglion ; its posterior end abutting on the antero-dorsal surface of the ganglion. Fig. 7, Plate LIII, shows a vertical section of the eye and ganglion, nearly in the direction of the long axis of the eye. Fig. 8, Plate LIII, represents a cross-section of the basal portion of the eye. The relative position of the histological elements is much the same as in Salpa cylindrica. In the posterior portion of the eye the rod cells are dorsal and the pigment cells ventral. In the anterior portion this arrangement is reversed, the pigment being dorsal and the rod cells ventral. The innervation is as in Salpa cylindrica. The optic nerve arises from the antero-dorsal part of the ganglion, running over the dorsal face of the posterior part of the eye, innervating the rod cells of this region, and then bores through the eye to innervate the thin-walled ends of the ventrally directed rod cells of the anterior portion. The structure of the rod cells is the same as in Cyclosalpa pinnata or Salpa cylindrica. The pigment cells are fewer in number than in either of the latter species and are not massed together. They are branched, having an appearance as if amœboid. They are large, with large nuclei and finely granular protoplasm in which is found a great quantity of pigment granules of different sizes. The intermediate layer of the retina is present and the cells are remarkably distinct. Fig. 8, Plate LIII, shows their appearance. They are small cells with small nuclei. Their protoplasm is clear and almost homogeneous, like that of the nucleated ends of the rod cells. The most interesting feature is that they each send up one (or more?) processes toward the rod cells. Although I have been unable to make successful macerations and cannot speak with absolute certainty, still I am convinced, after careful study of serial sections, that each of these processes connects with the protoplasmic core of the thick-walled end of one of the rod cells. Fig. 8 shows that the delicate membrane that surrounds the eye does not include the pigment cells, but intervenes between them and the intermediate cells. The pigment cells are attached, some to this membrane, and some to the basement membrane of the ectoderm, while others are attached to both. A similar, though less distinct membrane is found between the intermediate cells and the pigment cells in certain other species : e. g., Salpa cylindrica, solitary form ; Salpa hexagona, solitary and chain forms, and Salpa cordiformis-zonaria, solitary form. Also in several species, where the intermediate cells are not present, the rod cells are separated from the pigment by a similar membrane.

Beside the larger eye there is, in the chain form of Salpa runcinata-fusiformis, a smaller collection of cells just dorsal to the point of origin of the optic nerve, which have a structure closely resembling the structure of the rods in many species. Fig. 7, a vertical longitudinal section, and Fig. 9, a cross-section of the ganglion, show the structure of these cells (ex). Like the rod cells of the solitary form of this species, they have no regular shape, but the characteristic thickening of their cell walls is present. As will be seen by comparison with forms yet to be described, they must be regarded as rod cells which are either degenerate or have not attained to the typical structure. Their position, dorsal and posterior to the point of origin of the optic nerve, indicates that they are homologous with the smaller eye found in the chain Salpa cylindrica, and so with the dorsal pair of small eyes found in Cyclosalpa pinnata, chain form.

SALPA AFRICANA-MAXIMA.

The shape of the eye of the solitary form resembles that found in the solitary Salpa runcinata-fusiformis. (I have been unable to study sections of the solitary form.) In histological structure the large eye of the chain form agrees very closely with that of the chain form of the last species (Fig. 1, Plate LIV). There is a mass of imperfect rod cells in the dorsal part of the ganglion corresponding closely in shape, in position and in the character of its component cells to the similar structure found in Salpa runcinata-fusiformis. The only noticeable difference between the eyes of the two species is in the shape of the larger eye in the chain forms. In Salpa africana-maxima this is merely globular or slighly ovoid, while in Salpa runcinata-fusiformis it is elongated ovoid. The close resemblance between the eyes of these two species indicates the closest relationship.

SALPA HEXAGONA.

The Fish Commission collections contain no specimens of the solitary form of this species. The principal eye of the chain form much resembles the larger eye of the chain Salpa runcinata-fusiformis in shape, relative position of histological elements and innervation. (Compare Fig. 5, Plate LVII, with Fig. 6, Plate LVI, and Figs. 13 and 14, Plate LII, with Figs. 7 and 8, Plate LIII.) The histological character of the rod cells resembles more closely that of the imperfect rod cells of the small

eye of the chain Salpa runcinata-fusiformis, and yet more closely that of the rod cells of the eye of the solitary form of that species. The rod cells are roughly cylindrical, with irregularly thickened walls. They are not arranged in a single definite layer, but are somewhat irregularly disposed. In the cross-section (Fig. 14, Plate LII) we see the nerve fibers, *on*, on the dorsal surface; then an irregular layer of rod cells, *s, r*; in some places this layer is several cells deep. The dorsal ends of these cells are thin-walled; the ventral ends are thick-walled. Ventral to the thick-walled ends of the rod cells is a considerable number of nuclei, *i*, the nuclei of the intermediate cells. The rod cells and intermediate cells are enclosed in a very delicate membrane. Outside this membrane, ventral to the intermediate cells, between them and the ectodermal optic sheath, are the pigment cells, resembling the pigment cells of Salpa runcinata-fusiformis, chain form. They are irregular in shape; their protoplasm is so full of pigment granules that their nuclei can be seen only after dissolving the pigment in acid. Fig. 14 represents a section of the basal portion of the eye. A section of the apical portion would show the same appearance, with the exception that the dorsal and ventral sides of the section would be reversed.

In the chain form of Salpa hexagona there is a pair of dorso-lateral outgrowths from the ganglion, one on the right side, the other on the left side of its dorsal face (Fig. V, Plate LVII, and Fig. 15, Plate LII). These are spherical and composed of polyhedral cells with thick walls and large nuclei, of the size of the large nuclei found in the periphery of the ganglion or in the rod cells of the dorsal eye. The character and homology of these outgrowths can best be discussed after describing a simpler form of what I regard as the same organs in Salpa scutigera-confederata. I will now call attention only to the fact that their position on the dorsal surface behind the optic nerve corresponds to the position of the smaller eye of the chain Salpa cylindrica or Salpa runcinata-fusiformis, and the character of the cells of the outgrowths resembles that of the imperfect rod cells of the smaller eye of the latter species.

Professor Brooks has called my attention to a peculiar structure in the young embryo of Salpa hexagona. It is seen in the embryo at a time when the ganglion is well formed but has not yet attained the compact structure seen in the latest stages; the central cells of the ganglion have not yet commenced to degenerate nor has the eye appeared. The ganglion lies close to the dorsal ectoderm. Near the posterior end of the ganglion there is a wide and deep invagination of the ectoderm,

running obliquely downward and backward toward the dorsal wall of the cloaca (Plate LVII, Figs. 1 and 2). The walls of the invagination are pressed together so that the whole structure appears to be merely a double fold of ectoderm with no lumen between. Fig. 4, a diagrammatic view from the dorsal surface, shows the general outlines. The double fold of ectoderm is crescent-shaped, the horns of the crescent projecting forward on each side of the ganglion a little beyond its middle point. It is wider than the ganglion and a little longer antero-posteriorly. Figs. 1 and 2, longitudinal and cross-sections respectively, show that the ectodermal fold runs down toward the wall of the cloaca but does not quite reach it. It approaches the cloaca and not the pharynx, since its posterior end is behind the junction of the gill and the dorsal wall. In histological character the cells of the invagination resemble the ectodermal epithelium. This structure arises as a mere pit in the ecto-derm, following the lateral and posterior boundaries of the ganglion (Plate LVII, Fig. 3). In the earliest stages, when the ganglion is just commencing to enlarge, the dorsal ectoderm is an even surface. As the ganglion grows it pushes the ectoderm upward, forming a rounded hillock. In later stages of development the ectodermal epithelium still clings to the whole dorsal surface of the ganglion. That portion of the ectoderm behind the brain, however, grows rapidly and pushes up over the brain, overlapping from behind forward the epithelial covering of the brain. In this way there is formed the double fold of ectoderm described. It arises, then, not as a direct invagination, but by an over-growth. I do not know its fate, since I have had no opportunity to study the later stages of development or the adult of the solitary form. I am unable to see that this structure has any morphological or physiological significance. It does not seem to be worthy of the name organ, though possibly, if present in the adult, it may serve as a slight protection for the ganglion.[1]

SALPA COSTATA-TILLENII.

In the chain form of this species (Plate LVI, Fig. 2), which shows a still less definite structure of the rod cells in the larger eye, there are

---

[1] Oka (13) describes peculiar ectodermal proliferations on each side of the hypo-physical tube in the buds of Botryllus, which have about the same position and appear at about the same stage of development as does the ectodermal invagination described in the embryo of Salpa hexagona. Neither structure is sufficiently well understood to justify a comparison.

indicated structures which I regard as probably homologous, one with the dorsal pair, the other with the posterior pair of smaller eyes of Cyclosalpa pinnata, chain form. Fig. 10, Plate LIV, shows a vertical section through the ganglion and eyes in the direction of the long axis of the eye. In the large eye is seen an irregular mass of rod cells which show no indication of the typical structure except in the thickening of their walls. They are irregularly polyhedral in form and are not noticeably elongated. There is but slight indication of two regions of the eye corresponding to the two regions in the larger eye of the chain form of Salpa runcinata-fusiformis. This indication is given by the arrangement of the pigment cells. There is a single continuous layer of these between the mass of rod cells and the ectoderm of the optic sheath. This layer is, however, thicker and more dense in two regions, one on the ventral face of the eye near the ganglion, p', the other on the dorsal face near the apex, p''. The positions of these more developed portions of the pigment layer correspond to the positions occupied by the two masses of pigment cells in the two regions of the larger eye in the chain forms of Salpa hexagona, Salpa runcinata-fusiformis and other species. In this way we have a slight, but decisive, indication of the division of this eye into two regions which, when typically developed, show the structure found in the larger eye of the chain form of Salpa runcinata-fusiformis.

On the posterior face of the ganglion there is another large mass of irregularly polyhedral cells with thickened cell walls, closely resembling the rod cells of the dorsal eye (Fig. 10, Plate LIV). What may be the meaning of these cells I am unable to say with certainty. The cells much resemble the rod cells of the large dorsal eye of the same species; the posterior pair of eyes in the chain Cyclosalpa pinnata, though much smaller, occupy about the same position; the pigment spots in the ganglion of pyrosoma are in a position corresponding to the ventral portion of this mass of cells. I think it very probable that this structure is homologically an eye, having the same relation to the posterior pair of small eyes found in Cyclosalpa pinnata that the large dorsal eye of Salpa costata-Tillesii bears to the unpaired eye of the former species. On the opposite side of the ganglion, beneath the dorsal eye and in front of the optic nerve, there is still another mass of similar, imperfect rod cells, *ex*, and, in close connection with them, a patch of deeply pigmented cells. Neither the rod cells nor the pigment cells show so well in the section figured as in the adjacent sections. The structure of this mass of cells and its position, comparable to the position of the dorsal pair of small

eyes in the chain form of Cyclosalpa pinnata, indicates that it is homologous with these eyes in the latter species. The presence of pigment in connection with this structure in Salpa costata-Tillesii is a variation which confirms the view that it is a light-perceiving organ, though the typical histological character of the rod cells is not found.

We come now to quite a distinct group of salpas, including Salpa scutigera-confederata and another species, or more probably variety, not recognized by Traustedt, which is probably the Salpa bicaudata of Salensky and various authors.

SALPA SCUTIGERA-CONFEDERATA.

I have not been able to section the eye of the solitary form of this species, for the Fish Commission collections included but one specimen. In surface view (Plate LVI, Fig. 7) the eye almost exactly resembles that of the solitary Cyclosalpa pinnata, i. e., it is typical.

The dorsal eye in the chain form (compare Fig. 1, Plate LVI, which represents the very similar eye of the chain Salpa bicaudata) shows two well-marked regions, in one of which (the anterior) the pigment is dorsal and the rod cells ventral, while in the other (the posterior) this arrangement is reversed. The rod cells are of the same character as those of the larger eye of the chain Salpa costata-Tillesii (compare Fig. 2, Plate LV, with Fig. 10, Plate LIV). The pigment cells are so massed together and so full of pigment that no structure can be made out. The optic nerve arises from the dorsal part of the ganglion and enters the eye between the pigment layer of the posterior region and the rod cells of the anterior region. No intermediate cell layer can be distinguished. The greater share of each region of the eye (all save the pigment layer) is composed of thick-walled, polyhedral cells like those in Salpa costata-Tillesii, chain form. The comparative thickness of the cell walls is greater, however, in Salpa scutigera-confederata.

Two masses of similar, thick-walled cells are present in the ganglion, one on the right, the other on the left, a little above the mid-point of the lateral faces of the ganglion (Plate LV, Fig. 5, y). These cells exactly resemble the peculiar rod cells of the large dorsal eye in size, shape, character of nuclei, thickness of cell walls, in manner of staining, and in their general appearance. The arrangement of the chromatin in their nuclei and in the nuclei of the rod cells of the dorsal eye is very different from that seen in the other cells of similar size found in the

periphery of the ganglion. In the former, the nuclei contain many small chromatin granules, and no very large nucleolus. The other cells of the ganglion are of two sorts, the one sort small, with small nuclei. These are utterly different from the cells we are discussing. The other kind of ganglion cells is larger, about equal in size to the rod cells of the eye. They have the same sized nuclei, but in these the chromatin is nearly all collected into a large nucleolus, giving a decidedly different appearance from the nuclei of the rod cells. Besides this their protoplasm reacts much more strongly with hæmatoxylin, giving a deep stain, while the protoplasm of the rod cells stains very weakly. These characters, and especially the great thickness of their cell walls, distinguish the rod cells of the eye and of the two lateral masses in the ganglion from any others of the nerve cells. We must, then, regard these two lateral masses of rod-like cells that are found in the ganglion as imperfect or degenerate eyes, bearing the same relation to the larger eye of this species as the smaller dorsal eyes of the chain Cyclosalpa pinnata do to the large, unpaired eye of that species. These structures just described form a connecting link between the smaller eyes found in Cyclosalpa pinnata and Salpa cylindrica, which are undoubted optic organs, and other structures found in Salpa hexagona,[1] Salpa costata-Tillesii, and Salpa cordiformis-zonaria, which are so different from the typical eye that one would not readily recognize their true character. These structures cannot function as optic organs, and when I apply to them the word eye I mean merely that they are homologous to structures in other species that are undoubted eyes, and that the same sort of histological modification which has produced them from the cells of the ganglion has produced the rod cells of the larger dorsal eye of Salpa scutigera-confederata.

The manner of innervation of the dorsal eye of Salpa scutigera-confederata is very suggestive when compared with that of the corresponding eye in the species thus far described. In this species the optic nerve passes up from the ganglion and enters the eye at its *mid-ventral point* (Fig. 2, Plate LV). This reminds one strongly of the condition in the very young chain Cyclosalpa pinnata (Fig. 7, Plate XLVII), where the eye has the form of a thickened disk close pressed to the ectoderm, and the

[1] The lateral outgrowths from the ganglion of the chain Salpa hexagona are similar in structure and position to the two lateral masses of rod cells in Salpa scutigera-confederata and seem clearly to be homologous with them (compare Fig. 5, Plate LV, with Fig. 15, Plate LII).

fibers of the optic nerve bind the center of its ventral face to the dorsal part of the ganglion. This primitive condition, which has been retained in Salpa scutigera-confederata, has been altered in Cyclosalpa pinnata, owing to the reversal that takes place during the development of its eye, by which the originally anterior edge of the optic disk becomes posterior and its originally ventral surface dorsal. In this way, in Cyclosalpa pinnata, the optic nerve came to lie on the dorsal face of the posterior part of the adult eye. A comparison of the figures on Plates XLVII and XLVIII will show the manner of this change. A comparison of Figs. 1, Plate LIII; 4, Plate LII; 7, Plate LIII; 1, Plate LIV; 13, Plate LII, and 10, Plate LIV, will show that the manner of innervation of the dorsal eye of the chain Cyclosalpa Chamissonis, Salpa cylindrica, Salpa runcinata-fusiformis, Salpa africana-maxima, Salpa hexagona, and Salpa costata-Tillesii agrees closely with that found in the chain Cyclosalpa pinnata. This can be explained only by the supposition that in all these species there has occurred a shifting during the development of the eye, comparable to that described for Cyclosalpa pinnata. The innervation of the dorsal eye of Salpa scutigera-confederata shows that in this species no such shifting of the eye has occurred. The portion (anterior) marked e' in Fig. 1, Plate LV, is, then, homologous to the portion (posterior) marked e' in Fig. 1, Plate XLIX, and the portions marked e'' in the two figures are homologous. The eye of this species is, then, in this regard, more primitive than that of the others. We can say that in respect to position and innervation the dorsal eyes of the chain forms of the other species just mentioned pass through a scutigera-confederata stage.

SALPA BICAUDATA (?).

The dorsal eye of the chain Salpa bicaudata (Fig. 1, Plate LVI) is very closely related to that of Salpa scutigera-confederata, chain form. It presents, however, points of difference sufficient to indicate at least a varietal distinction between this and the latter species. The eye is strongly bilobed (Plate LV, Fig. 6); the two lobes representing the two regions (anterior and posterior) seen in the dorsal eye of the chain Salpa scutigera-confederata. There is seen the same contrast in the relative position of the pigment layer and the rod cells in the two lobes as in the two regions of the eye of the last species. The two lobes of the eye are not, however, anterior and posterior, as we would expect. A twisting through an arc of forty-five degrees has taken place, by which they are

brought to lie in a direction oblique to the long axis of the body. The innervation of the eye is the same as in the last species, except that the optic nerve forms a long stalk by which the eye is elevated above the ganglion. Fig. 7, Plate LV, shows this stalk and one lobe of the eye. The section is vertical and nearly longitudinal. Fig. 6 shows a section through the base of the two lobes of the eye and the optic nerve between them. In Salpa bicaudata there are no smaller eyes, nor any indication of structures homologous to them.

Above the eye in the chain form of this species there is a decided thickening of the tunic, forming a cushion, or pad, that must be a very effective protection for the eye and ganglion.

I have before referred to the nucleus-like bodies in the thick-walled ends of the rod cells of Cyclosalpa pinnata. In Salpa bicaudata similar bodies are seen in the center of the rod cells; the true nuclei of the cells lying near the periphery (see Fig. 6, Plate LV). In this species they are unusually large. They stain more deeply than the protoplasm and less deeply than the cell walls. They are indicated in Fig. 6 merely in outline, the deeper staining not being represented.

SALPA DEMOCRATICA-MUCRONATA.

In Salpa democratica-mucronata we have decided points of difference from any other species. The eye of the solitary form (Fig. 7, Plate LVII) closely resembles that of Salpa cylindrica, as a comparison of Figs. 10, 11 and 12, Plate LV, with Figs. 1, 2 and 3, Plate LII, will show. The chief point of difference is that in the former there are no intermediate cells of the retina to be distinguished. There is also a slight difference in shape, since in Salpa democratica-mucronata there are three distinct swellings of the rod cell layer of the retina, as shown in Plate LVII, Fig. 7. One of these is posterior on the median line, the other two on the two anterior horns of the horseshoe.

In the chain forms of all other species the larger eye is situated on the dorsal or antero-dorsal face of the ganglion. In the chain Salpa democratica-mucronata it is placed on the antero-ventral face of the ganglion (Plate LVII, Fig. 6, a dorsal view). The form and structure of the eye and the arrangement of the ectodermal optic sheath show that this peculiarity of position is due to a shifting of the whole ganglion, by which the originally dorsal face has become antero-ventral. The eye then arises from what was originally the dorsal face of the ganglion.

The ectoderm has remained attached to the originally dorsal and anterior faces of the ganglion, as it is in other species; so, in the shifting of the ganglion, it has been carried ventralward, making a loop beneath the ganglion. A comparison of Fig. 14, Plate LV, with Fig. 2, Plate LV, makes this intelligible. The points marked *an* in each are homologous and were both originally anterior (compare also Fig. 10, Plate LIV). If we bear in mind this change of position it will render the description of the eye itself much clearer.

The eye is divided into three portions, *e'1*, *e'2* and *ex* of Figs. 14 and 15, Plate LV (see also Fig. 6, Plate LVII). In the anterior of these, *ex*, the rod cells are on the side toward the ganglion, *i. e.* are dorsal, and the pigment layer is next the ectoderm, *i. e.* away from the center of the ganglion or ventral. In the other two portions the rod cells point away from the ganglion, *i. e.* are ventral. In one of these two portions (*e'1*) the rod cells point more forward, while in the other portion (*e'2*) they point nearly ventralward (compare Figs. 14 and 15, Plate LV, and Fig. 6, Plate LVII). The optic nerve enters the eye between the three regions and is distributed to their rod cells, as seen in Figs. 14 and 15, Plate LV. If, now, Fig. 15 be revolved through an arc of 180° in a plane vertically perpendicular to the paper and it be compared with Fig. 4, Plate LII, we see that we have the following relation between the two, shown by the arrangement of rod and pigment cells and by the manner of innervation. The portion marked *ex* in one is homologous with the portion marked *ex* in the other; the only difference being that in Salpa democratica-mucronata this portion has pushed out a little further from the ganglion. The portions marked *e'1* and *e'2* in Fig. 15, Plate LV, correspond to the single portion marked *e'* in Fig. 4, Plate LII, *i. e.*, they correspond to the undivided basal portion of the large eye of the chain Salpa cylindrica, and so to the two posterior limbs of the larger eye of Cyclosalpa pinnata, chain form. There is in Salpa democratica-mucronata no portion of the eye homologous to the apical portion, *e''*, of the large eye of the chain Salpa cylindrica or Cyclosalpa pinnata. These homologies are deduced from three points: first, the evident shifting that has taken place in the ganglion of Salpa democratica-mucronata; second, the relative position of the histological elements in the different portions of the eye, and the relation of this position to the three principal axes of the ganglion; third, the innervation of the eye.

We see, then, that Salpa democratica-mucronata falls into the same group with Salpa cylindrica, Salpa runcinata-fusiformis, Salpa Africana-

maxima, Salpa hexagona and Salpa costata-Tillesii, and that in this species, as in those, there has occurred a shifting of the developing eye of the chain form that has caused the originally ventral surface to become dorsal, as was seen in the development of the chain Cyclosalpa pinnata, and is shown by the innervation to have occurred in the development of all the members of the group just mentioned.

SALPA CORDIFORMIS-ZONARIA.

This species shows quite aberrant features in the eye of both the chain and solitary forms. The eye of the solitary form agrees in histological character with the eye of the solitary Cyclosalpa pinnata or Salpa cylindrica; i. e., it has the typical histological structure. (See Figs. 14 and 15, Plate LIV.) In shape, however, it shows a divergence from the eyes of all other species. The anterior ends of the horseshoe-shaped eye project dorsalward and obliquely to the right and left (Fig. 5, Plate LVI). Only the posterior curved part of the eye rests on the surface of the ganglion. Although the shape is different from that of the typical eye of the solitary form, it is really but a slight modification of that eye, the anterior limbs of the horseshoe having been elevated above the ganglion, losing contact with it. It is important, however, as the only considerable modification shown in the shape of the eye of the solitary form of any species of salpa or cyclosalpa.

In the chain form the eye has the typical position on the dorsal face of the ganglion (Plate LVI, Fig. 3 ; Plate LIV, Figs. 11, 12 and 13). It is oval in shape, with its long axis a little inclined to the long axis of the body. A study of the histological structure and the arrangement of the histological elements shows that here again we have represented, in a disguised form, the anterior and posterior regions of the eye, comparable to the similar regions found in the large eye of the chain Salpa runcinata-fusiformis and others. Fig. 13, Plate LIV, shows the condition found. It represents a vertical section in the direction of the long axis of the eye, a little on one side of this axis. The rod cells of the typically basal portion are seen at s'. They are seen to connect directly with the fibers of the optic nerve. The rod cells of the typically apical portion are seen at s''. The eye of this species is very compact; the apical portion being brought back under the basal portion, so that the thick-walled ends of the rod cells of one region are in close connection with the thick-walled ends of the rod cells of the other region. In Fig. 13 we see the pigment

of the typically apical portion. In a section exactly in the mid-line of the eye (Fig. 12, Plate LIV) we would see that the apical portion of the eye does not there approach so far toward the base of the eye, and that beneath the rod cells of the basal region are pigment cells like those seen in the section drawn. This eye, then, in spite of its considerable modification, still conforms to the usual plan ; and its manner of innervation exactly resembling that found in the large eye of the chain Salpa runcinata-fusiformis, shows that in this species also we have a reversal of position in the developing eye. The rod cells are columnar, with their outer nucleated ends thin-walled and the cell walls of their inner ends unevenly thickened.

It may seem that possibly the irregular shape of the rod cells seen in the sections of the eyes of the chain individuals of certain species, e. g., Salpa hexagona, Salpa costata-Tillesii, Salpa cordiformis-zonaria, is due to the cells being cut obliquely. I would say that I have cut sections of each eye in three planes and find the same irregularity of shapes in all.

I realize the incompleteness of the histological observations given in the preceding pages. I cannot, however, hope to make them more complete until I am enabled to study fresh material by maceration methods, the use of which is so essential for gaining a right understanding of the shape of the cellular elements, and especially of the manner of innervation. I hope at some future date to make a more complete contribution to the knowledge of the histological elements of the nervous system and eye of salpa.

<center>THEORETICAL.</center>

We have seen in the foregoing descriptions that the eye of the solitary form has a very uniform structure throughout the different species ; the condition in Salpa cylindrica being probably typical. There are but two noteworthy variations: the first is the imperfect development of the rod cells in Salpa runcinata-fusiformis ; the second is the slight departure from the typical horseshoe-shape which is found in Salpa cordiformis-zonaria.

We have also seen that the eye of the chain form passes through a stage in its development when it resembles the eye of the solitary form. This is observed even in the group with the most modified eye, the cyclo-salpas.

In the chain forms of the different species there is a very great degree of variation in the structure of the eyes ; yet it is not difficult to

see, in all, distinct indications of the same fundamental plan, which is probably best exemplified in the chain Salpa runcinata-fusiformis. The variation affects the number, position, size, shape and histological character of the eyes; and so universal is the variation that the eyes of no two species are alike. Salpa runcinata-fusiformis and Salpa Africana-maxima most closely resemble each other, but even between these species there are differences in the shape of the larger eye, the size of the optic chamber, and the shape of the intermediate and pigment cells.

This so great and so prevalent variation in the eyes of the several species, which yet conform more or less closely to the same fundamental type, offers an especially favorable opportunity for studying the relationship between species. If the variation had been so great that the conformity to the type were lost in the adult, a careful embryological study of the eye would be necessary to throw much light upon the relationship; but, since in the adult forms we have present both the variation and the fundamental conformity, we can safely deduce certain evidence as to relationship from a comparative anatomical study. This, in connection with a careful study of the development of the most highly specialized eye (that of Cyclosalpa pinnata), gives us good data from which to gain evidence as to the phylogeny of the different species of salpidæ. The history of a single important organ which we have in this way obtained can be reasonably taken as a source of definite and important evidence as to phylogeny; though, of course, it is only evidence and cannot be taken as decisive proof. A similar study of all the organs would be necessary to fully establish the phylogenetic relations between the species.

In salpa we are especially fortunate, since in the solitary form we have a conservative member, while in the chain form we have a more modified member of the species. It is as if an animal were placed in such peculiar conditions that one portion of its body should retain primitive characters while another portion of its body should undergo the most decided changes. In salpa the divergent member (chain individual) is so removed from the more conservative member (the solitary salpa) that its modifications have unusually little effect upon many features of this conservative member. The modifications of the chain form do not, however, have any less bearing upon phylogenetic questions than if they also affected the solitary form of the species. Variations in the chain form and variations in the solitary form all come from the same ultimate source, the germ plasm produced by the solitary salpa.

What relationships between species does this evidence from the eyes favor?
First, the fundamental uniformity of plan in the eyes of the different
species shows what is universally recognized, namely, a common ances-
try; second, we see certain instances of the closest agreement in the
structure between the eyes of two or more species, indicating a natural
group. Cyclosalpa pinnata and Cyclosalpa Chamissonis form one such
quite distinct group. The eye of the chain Cyclosalpa pinnata passes
through a Chamissonis stage; indicating that the latter species is the
more primitive of the two. Again, the almost perfect uniformity of
structure in the eyes of Salpa runcinata-fusiformis and Salpa Africana-
maxima indicates that these two form a natural group. But the fact
that the manner of innervation of the large dorsal eye of the chain form
of these species points to a reversal of position during the development
similar to that described for Cyclosalpa pinnata, indicates that these two
salpas fall into a larger natural group with the cyclosalpas. This group
includes also the other salpas in which a similar reversal of the position
of the eye has taken place: *i. e.*, Salpa cylindrica, Salpa hexagona, Salpa
costata-Tillesii, Salpa cordiformis-zonaria and Salpa democratica-mucro-
nata. Quite distinct from this large group is another natural group
including Salpa scutigera-confederata and Salpa bicaudata, in which no
reversal of the eye has taken place. This is shown by the manner of
innervation. The large eye of the chain Cyclosalpa pinnata passes
through a stage when in shape, position and innervation it resembles
the adult eye of the chain Salpa scutigera-confederata; so it is safe to
say, so far as the evidence from the study of the eyes goes, that the group
including the latter species is more primitive than the large group in
which we find Cyclosalpa pinnata and Salpa runcinata-fusiformis. The
imperfect condition of the rod cells of Salpa scutigera-confederata and
Salpa bicaudata indicates perhaps that these forms have somewhat
degenerated from the ancestral type, to which, however, they are more
closely related than any other species so far as regards the shape,
position and innervation of the eye.

Between the members of the larger group we find varying degrees
of resemblance in the eye. Salpa cylindrica stands about midway
in the group. Salpa democratica-mucronata shows the greatest diverg-
ence from this species. Salpa runcinata-fusiformis and Salpa Africana-
maxima agree quite closely with it. Salpa cordiformis-zonaria stands
quite far removed. The Cyclosalpas form a distinct group with a
more highly specialized eye than Salpa cylindrica, yet bearing a close

relation to that of the latter. Salpa costata-Tillesii shows features of resemblance to each of the members of the cylindrica group, and at the same time shows features of difference from each. It is hard to say to which it is most closely related in the structure of the eyes, though in the position of its smaller eyes it most nearly approaches the cyclosalpas.

The relationships toward which the comparative anatomy of the eyes of the different species would point are graphically expressed in the following table. The reason for placing Salpa Africana-maxima as the most primitive of the cylindrica group will appear when we take up the subneural gland.

DIAGRAM 1.

C. pinnata.
|
C. Cham.

S. cyl.

S. hex.   S. cost.-Till.

S. dem.-muc.   S. cord.-zon.

S. runc.-fus.
S. Af.-max.

S. bicaud.
S. scut.-conf.
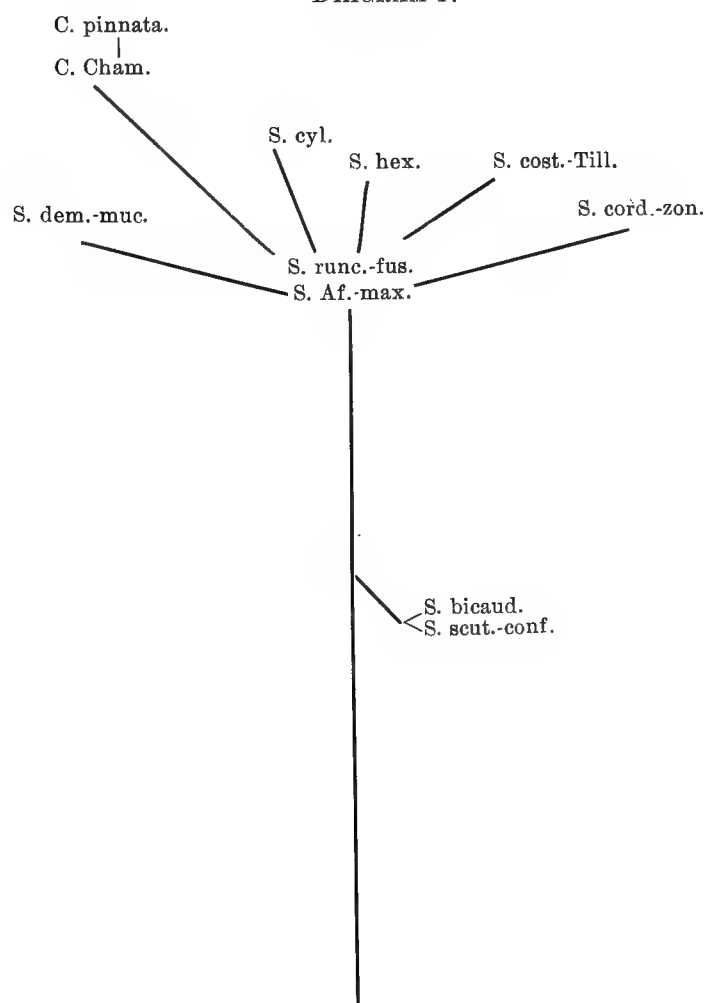
This table does not pretend to show the actual relation between species, but rather that relation toward which the evidence derived

solely from a comparative study of the eye tends. In order to determine the actual relationships it would be necessary to determine the relationships indicated by the comparative study of each of the systems of organs and then to compare results. The study of the eye does, however, offer especially favorable conditions for judging of relationships between species, and the evidence from this source is worthy of emphasis.

What is the fundamental plan of the eye of salpa?

The eye is formed from the central nervous system and not directly from the ectoderm, conforming in this respect to the vertebrate rather than the invertebrate type. It is a simple eye, readily comparable in structure to the pineal or lateral eye of vertebrates. It is composed of a series of optic units, each of which consists of a rod cell and one or more pigment cells. That end of the rod cell which receives the innervating fiber is thin-walled and contains the nucleus. The other end of the rod cell which is near the pigment cells is thick-walled, resembling the thick-walled ends of the vertebrate rod and cone cells. The following diagram will show the relation between the structure of the salpa eye, C, and that of the vertebrate lateral or pineal eye, D.

The resemblances are seen to be considerable. The rod cells, the essential element in each, are alike, and the innervation is the same. The optic unit of the vertebrate eye is more complex than that of salpa, having certain ganglion cells interpolated between the rod cells and the brain; but the fundamental character of the two is the same. The eye in the two groups is formed by a similar modification of the cells of the central nervous system, this modification having gone further in the vertebrate than in salpa. *Although the eye of salpa so closely resembles the vertebrate eye in structure, it cannot be regarded as homologous with the latter.* It has often been homologized with the ascidian larval eye and the vertebrate pineal eye, but such an homology cannot be sustained, as we will see after we have studied the relation of the ascidian nervous system to that of salpa. We will see that the eye of salpa is derived from a portion of the nervous system not represented in the ascidian tadpole or in vertebrates. This will be taken up in connection with the study of the subneural gland.

We have in the chordata a series of variations in the *structure* of the optic organs. Salpa eye falls into this series, although it is not homologous to the optic organs of any other chordate. In amphioxus we find merely a slight pigmentation of the inner ends of certain cells in

the anterior part of the neural canal (Diagram 2, A). In the larval ascidian we find a number of cells in a corresponding position, whose inner ends abut on a mass of pigment (Diagram 2, B). These cells are slightly evaginated, and in the hollow of the evagination there are one or more "lenses,"[1] each of which has been formed by the migration of one of the neural cells into the hollow of the optic evagination. In the vertebrate pineal eye (Diagram 2, D) we have this evagination carried much further and the lens formed in a different way, but the rod cells correspond to those of the larval ascidian eye in their relation to the neural canal, their pigmented ends being toward the cavity of the neural tube, or rather of the outgrowth from the neural tube. Salpa eye (Diagram 2, C) forms a connecting link *in structure* between the larval ascidian eye and the vertebrate pineal eye, and though it is formed in a different way from a different part of the brain and forms no part of the phylogenetic series, still it indicates a probable phase in the structural development of both the pineal and lateral eye of vertebrates.

DIAGRAM 2.

A = optic unit of amphioxus eye (?).

B = optic unit of clavelina eye.

C = optic unit of salpa eye.

D = optic unit of vertebrate lateral or pineal eye.
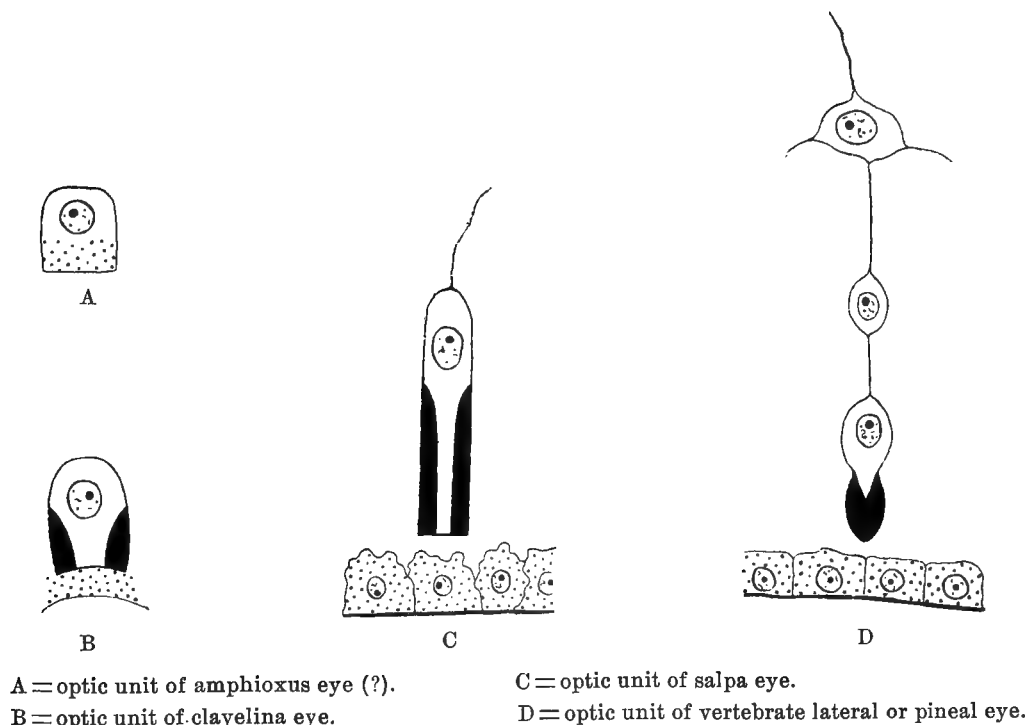
In the optic unit of certain species of salpa we find a cell interposed between the rod cell and the pigment cells. I refer to the intermediate

[1] I have often found several such lens-like bodies in the eye of clavelina surrounded by the mass of pigment granules.

cells, as I have called them. These cells are not constant, being present in some species and absent in others, and in certain species being present in the solitary form and absent in the chain form. I do not think they can be regarded, in every case where present, as simply certain of the cells of the pigment layer which have not become pigmented. In some cases this may be the explanation, but in Salpa runcinata-fusiformis they are separated from the pigment layer by the definite membrane which encloses the rod cells, and also they send up processes toward the rod cells which appear to connect with the protoplasmic core of the thickened ends of the rod cells (Plate LIII, Fig. 8). What may be the meaning or function of these cells I am unable to say.

The variation found in the eyes of salpidæ is itself an interesting feature. The fact that this variation is slight in the solitary forms while it is very great in the chain forms is an important fact. So far as we now know, there is nothing in the conditions of life of the chain forms which would create a greater need for eyes of diverse structure than is found in the solitary form. It may be that there is less rigorous selection of optic organs in the chain individuals, owing to the interdependence and mutual assistance of the members of the community.

We have noticed the great variety of shape, position and histological structure shown by the larger eye of the chain individuals of different species. An even more remarkable series of variations is shown in the smaller eyes. In some species, e. g., Salpa cylindrica, Cyclosalpa pinnata, these show the normal optic structure. In other species, e. g., Salpa hexagona, Salpa scutigera-confederata, they bear no resemblance to eyes, yet the gradations between the two conditions shown in other species, e. g., Salpa runcinata-fusiformis and Salpa costata-Tillesii, seem to prove beyond question that all these structures are homologous. Because of this homology I have called them all eyes, not meaning that they all function as visual organs. What is the meaning of this great diversity of structure I am unable to say. If it was not for the embryological evidence to the contrary we would seem to be dealing with a series of degeneration. If this be so I am unable to conceive what change in the life conditions of the chain individuals has caused them to have less need of numerous, well developed optic organs than formerly. It is useless to speculate upon the matter.

We have seen that in the most primitive form of salpa eye (that found in the solitary individuals) the thick-walled ends of the rod cells and the pigment layer are normally toward the brain (or, what is essen-

tially the same thing, toward the core of the optic ridge), while their innervated ends are near the surface. The light then traverses the whole length of the rod cells before reaching the pigment; this eye is therefore "inverted."[1] In the eye of the solitary form of Cyclosalpa pinnata a secondary shifting of the retina has taken place by which it is changed from the dorsal to the inner surface of the optic ridge. (Compare Figs. 7 and 8, Plate LI.) In the solitary form of other species the same process is carried still further, till the thin-walled ends of the rod cells lie toward the brain, and their thick-walled ends and the pigment layer lie just beneath the ectoderm (Fig. 1, Plate LII, and Fig. 14, Plate LIV). In these species the eye of the solitary form is therefore secondarily non-inverted. This change from the inverted to the non-inverted condition, shown in the ontogeny, is probably of phylogenetic significance.

In the ontogeny of the eye of the chain form we see a similar change effected in a somewhat more complicated manner. While the developing eye of the chain Cyclosalpa pinnata is still a disk of cells appressed to the ectoderm and connected to the brain only by the nerve fibers entering its mid-ventral point (Plate XLVIII, Fig. 1), we can distinguish the three regions of the eye (e', e'', e'''), and can determine the position of the as yet imperfectly developed histological elements. In the first region, e', the rod cells lie next to the brain and the pigment layer next to the ectoderm. This region of the retina is therefore non-inverted. In the second region, e'', the rod cells lie next the ectoderm and the pigment layer toward the brain; this portion of the retina is therefore inverted. In the later reversal of the eye, illustrated by the figures on Plate XLVIII, the relation of the whole retina to the ectoderm (i. e., to the morphologically exterior surface of the body) remains the same. The relation to the direction of the light is, however, exactly reversed. The first region, e', which was at first uninverted, becomes physiologically inverted; the second region, e'', which was at first inverted, becomes physiologically non-inverted. The changes in the third region of the retina, e''', are

[1] I have before described the *reversal* by which the eye of the chain individuals of nine species is turned bodily upside-down in the course of its ontogenetic development. I use the word *inverted* to refer to something wholly different, namely, the condition of an eye when the bacilli of its rod cells and its pigment layer are away from the source of light, while the protoplasmic, innervated ends of the cells are toward the light. This is the technical use of the word inverted and has no reference to what I have termed the reversal of certain salpa eyes. If it were not for the well-established technical usage it would be well to exchange the terms, but as it is, such a course would lead only to confusion.

still more complicated. Primitively it formed a portion of the second region, e'', and its histological elements had the same arrangement, i. e., were inverted. Later, when this third portion of the retina curled over backwards, revolving through an angle of 180° (compare Figs. 5 and 6, Plate XLVII, with Fig. 1, Plate XLVIII), it became secondarily uninverted. By the final reversal in the position of the whole eye this third region became again physiologically inverted. It is not easy to conceive the advantage derived from this complicated series of changes. One thing, however, seems to be most clearly indicated, namely, that there is no fundamental difference between inverted and non-inverted eyes, and that one sort can very readily and apparently for slight reason pass into the other sort. It is therefore to be expected that closely related animals may possess eyes differing in this regard.

A further comparison of the horseshoe-shaped eye of the solitary form with the immature eye of the chain individual should be made. That the two eyes are fundamentally similar is shown by the fact that the eye of the chain individual passes through a horseshoe-shaped onto-genetic stage. After it has assumed its disk-like form and before it has become reversed, its posterior portion (e'') corresponds to the posterior curved part of the horseshoe eye, and its anterior portion (e') to the two anterior limbs of the horseshoe. The primitive condition for the horse-shoe eye is that of inversion (as shown above); secondarily it becomes non-inverted. In the immature disk-like eye of the chain individual (Plate XLVIII, Fig. 1) the posterior portion (e'') has an inverted retina, i. e., is primitive in this respect, while the retina in the anterior portion (e') is from the first non-inverted; that is, the ontogenetic development of the second region of the eye (e'') retains more of the phylogenetic history than does the first region of the retina (e'). We must explain this as a cœnogenetic abbreviation of the ontogeny.

SECTION II.—*The Innervation of the Ciliated Funnel.*

The question of the innervation of the ciliated funnel in the ascidi-ans has been much discussed, but its actual innervation has never been shown. The nerve supply of the funnel in pyrosoma, doliolum and salpa has not, so far as I can learn, been demonstrated.[1] The fact of a definite nerve supply is essential to prove that it functions as a sense

[1] Joliet (7) argues plausibly that there is probably such a nerve supply in pyro-soma, but he does not show that it is present.

organ, a belief held by many of those who have worked upon the group. To establish this point, if true, one should study the living salpa, making physiological tests. He should also make teased preparations of macerated specimens. I have been unable to secure fresh material, and so could not study the innervation of the funnel in this way. I was able, however, to make out from a study of the preserved specimens some points that indicate a definite nerve supply.

The funnel and adjacent parts were removed from specimens that had been hardened in Perenji's fluid and preserved in alcohol. The portion removed was mounted in a mixture of glycerine and acetic acid, strongly colored with methyl green. This brought out clearly the nerve fibers, cell outlines and nuclei. In the chain form of Cyclo-salpa pinnata, on the dorsal surface of the walls of the ciliated funnel, there is seen in this way a highly developed plexus of branching nerve cells and fibers (Fig. 9, Plate XLIX). The plexus is closer (smaller meshed) over the posterior portion of the funnel, containing in this region more nerve cells and more fibers than are found further anterior. A pair of strong nerves run forward from the brain over the ciliated funnel and beyond it. These nerves pass directly through the plexus, and were seen in one or two cases apparently to give off very fine branches to the plexus. The actual branching of the nerves was not seen in section, for the nerves themselves are so small as to be traced with difficulty in serial sections, and the exceedingly fine branches would be impossible to find. The most careful focusing with high powers (1735 diameters) indicated that the appearance seen was an actual branching, and not the superposition of the nerve over the fine fiber, or *vice versa.*

In the solitary form of Salpa cordiformis-zonaria a similar, though less developed plexus is found over the posterior part of the dorsal tubercle. Two pairs of nerves, the one pair large, the other pair small, run forward from the brain toward the funnel. The outer pair pass over the plexus and beyond the funnel toward the mouth. The inner, smaller pair can be traced to the funnel and about one-third of the way over its dorsal surface, there disappearing. It seems probable that these nerves connect with the nerve plexus dorsal to the funnel, though the opacity of the ciliated funnel in this species rendered the region so difficult to observe that any actual connection was not made out. If there be such a connection the whole structure may be regarded as an apparatus for the innervation of the ciliated funnel. The pair of small

nerves seen in Salpa cordiformis-zonaria correspond probably to those fibers that leave the single pair of large nerves found in Cyclosalpa pinnata and join the plexus above the funnel. In other species of salpa these organs are developed to different degrees. (See Fig. 7, Plate LVI, and Fig. 7, Plate LVII.)

Lahille (10) has shown that a plexus of nerve cells and fibers is present over the pharynx wall and innervates the sensory cells of the lips. This manner of innervation is not, then, exceptional for salpa.

SECTION III.—*The Anatomy and Development of the Subneural Gland in Salpidæ, with incidental remarks upon the Homology of the Nervous System in different Groups of Tunicates.*[1]

So far as I can learn, the ciliated funnel is the only organ in salpa that has been regarded, up to the present time, as homologous with any portion of the subneural gland of ascidians. This has been universally regarded as homologous with the ciliated funnel of ascidians which serves in most species as the orifice of the excretory duct from the gland proper, and perhaps in some species as a sense organ also. There are present in salpa certain other structures that I regard as homologous with other portions of the subneural gland of ascidians, pyrosoma and doliolum. Before entering on the description of the condition in the adult salpas of different species I wish to review briefly the conditions found in the other groups of the tunicates, and then to take up the development of these organs in Cyclosalpa pinnata. After this a description of the gland in other species of salpa will be more intelligible. With this foundation we will then be able to discuss briefly the homology of the structures described in the different groups.

Among the ascidians clavelina, amarœcium, Phallusia mammillata and Molgula ampulloides will serve as examples.[2] In *clavelina* the ciliated funnel opens backward into a canal that lies upon the ventral surface of the brain, between the brain and the well developed subneural gland. This latter is traversed by small ramifying canals that open

---

[1] In a preliminary notice of this paper (12) I was led by a manuscript error to describe as cloaca that part of the branchial chamber ventral to the ganglion. A comparison of the adult condition with that found in the embryo leaves no doubt that it is really part of the pharynx.

[2] The description of the subneural gland in clavelina and amarœcium is based upon Miss Lilian Sheldon's paper (18).

inward into the large canal which is the duct of the gland. The duct and the small canals that run through the gland are lined by cubical epithelium. At the level of the anterior end of the ganglion this passes abruptly into the columnar ciliated epithelium of the funnel. At the line of demarcation between the two kinds of epithelium the dorsal wall of the duct is wanting, and the surface of the ganglion here bounds the lumen of the duct.

In the *adult amarœcium* the conditions are somewhat different. The duct is absent, the gland and ganglion coming into close contact; but the funnel is present and its posterior portion pushes between the front portions of the gland and ganglion. It opens freely to the former by a narrow opening, but there are no definite canals running through the gland and opening to the funnel. This is an indication of degeneration. The gland itself is degenerate; its walls having something the appearance of the vacuolated notochord cells in the chick embryo. The chief points of difference from the gland of clavelina shown by the gland of amarœcium are: (1) the degenerate condition of the gland; (2) the absence of any duct opening into the funnel; (3) the consequent absence of any canals opening from the gland into such a duct; (4) the fact that the surface of the ganglion does not at any point lie next to the lumen of the funnel, the single posterior opening from the funnel being toward the gland.

In the *larval amarœcium* the duct of the gland is represented by a posterior prolongation of the funnel that completely separates the gland and ganglion, reaching beyond their posterior limits. At one spot on the ventral wall of the duct and at an opposite point on its dorsal wall the epithelial lining is absent; the surface of the gland and ganglion at these points reaching the lumen of the duct. There are no canals running through the gland. The presence in the larval amarœcium of a duct of the subneural gland and of a communication between the ganglion and the duct show that the absence of these features in the adult amarœcium is due to degeneration.

In *Phallusia mammillata* Julin discovered certain other structures which throw great light upon certain organs in salpa I shall soon describe. The condition of the ganglion and duct is practically the same as in clavelina, but in addition to the small canals which open from the gland into the duct there are *many lateral canals connecting the duct with the peribranchial chamber*. Herdman (4) found an important variation in two specimens of Phallusia mammillata which he examined. In

these two individuals the communication of the gland with the ciliated funnel had been lost and the secretion of the gland could pass out only through the lateral communications with the peribranchial chamber.

In *Molgula ampulloides* Van Beneden (1) found the duct enlarged into two lateral chambers which are significant in this connection.

In *pyrosoma* the funnel is produced backward into a thin-walled canal that lies ventral to the ganglion, close pressed to its ventral surface. Seeliger (16) and also Lahille (10) figure a ventral evagination of the duct, with thicker walls than the rest, that seems to be homologous with the ascidian gland.

In *doliolum*, according to Uljanin (20), we find on the antero-ventral side of the ganglion a wart-like, solid process of nerve cells connected by a small hollow tube with a typically developed ciliated funnel. In the young doliolum the wart-like process from the ganglion contains a cavity of considerable size, which is continuous with the lumen of the funnel through the hollow tube. The cells of the tube in the young individual resemble the ganglion cells; but during the growth of the animal the tube elongates and, as its cells do not increase correspondingly in number, they are drawn out into flat pavement cells forming a thin, interrupted, epithelial wall around the slight central cavity of the tube. The neural membrane is continuous over the tube to the funnel, forming the basement membrane for the epithelial cells of the wall of the tube.

There are points in the *development* of the subneural gland and its duct in the ascidians, pyrosoma and doliolum that are of importance in connection with these organs in salpa. In pyrosoma and doliolum these organs are at first merely the anterior opening of the canal of the central nervous system into the pharynx. In doliolum the posterior portion of this canal is obliterated, but a portion of it persists till a late period in the wart-like, antero-ventral process from the ganglion.[1] The canal with its walls is represented in the adult by the process from the ganglion, the ciliated funnel and the hollow tube connecting them.

The canal of the central nervous system of the embryo pyrosoma opens freely to the pharynx.[2] This canal and its walls are represented in the adult by the ciliated funnel and its posterior prolongation that lies along the ventral surface of the ganglion, the ganglion being a secondary formation derived from the dorsal cells of the posterior part of the canal of the central nervous system. It arises at a comparatively late period by the proliferation of the cells of this region.[3] Accord-

[1] B. Uljanin (20).   [2] Oswald Seeliger (16), also W. Salensky (14).   [3] L. Joliet (7).

ing to Salensky, the primitive ciliated funnel and the duct connecting it with the brain atrophy, and later the definitive ciliated funnel arises at the spot where the primitive funnel at first appeared. The posterior end of the definitive funnel elongates till it comes to lie along the ventral surface of the ganglion, forming in the adult a hollow tube opening anteriorly to the ciliated funnel. It seems probable that the definitive funnel is merely the primitive funnel reopened, but be this as it may, the primitive funnel must probably be regarded as the homologue of the funnel in salpa and doliolum. This is shown by the perfect agreement in the three genera of the condition of these organs in the immature individual, and also by the greater or less degeneration of the communication between brain and funnel which occurs in all three genera subsequent to the time when they so closely agree in the structure of these organs. (See Appendix I.)

In the ascidians the early relation of the ciliated funnel and the duct that opens into it to the nervous system is difficult to determine, because of the contradictory statements of those who have studied these organs in the group. Kowalevsky (9) and others say the canal of the central nervous system at an early stage opens to the pharynx. From analogy with pyrosoma, doliolum and salpa this would be what we would expect. Van Beneden and Julin (1), however, deny the presence of such an opening, saying that the posterior prolongation of the ciliated funnel does not communicate with the canal of the central nervous system. According to these investigators the canal of the central nervous system in the larval ascidians is wholly distinct from the ciliated funnel. The nervous system is divided into three regions: (1) an anterior sense vesicle which atrophies in the later development; (2) a visceral portion from whose ventral cells the gland develops, while its dorsal cells give rise later to the adult ganglion; (3) a caudal portion which degenerates completely. In the later larval development, the posterior prolongation of the funnel, which is short in the earlier stages, elongates considerably, pushing between the ganglion and gland and becomes the efferent duct of the gland.

It is difficult to form an opinion whether in the early ancestors of the tunicates the canal of the then simple nervous system opened to the pharynx, and this connection was lost in the larval ascidian, owing perhaps to the great development of the anterior sense vesicle; or whether the ciliated funnel arose separately from the nervous system, as some claim it now does in the ascidians, and a communication

between the two was secondarily established. If the former hypothesis be the true one, pyrosoma, doliolum and salpa retain or have reverted to the primitive condition which the ascidian larva, according to Van Beneden and Julin, has lost. If the latter hypothesis be correct, the early appearance of the communication between the cavity of the nervous system and the pharynx in these three genera is an instance of accelerated development. (See Appendix I.)

Let us now turn to the *chain form* of *Cyclosalpa pinnata* and observe the development of the subneural gland and the organs connected with it. In an early stage of the development of the nervous system, long before any trace of the eye appears, the cavity of the brain and the lumen of the funnel open freely into each other by a wide duct, so short and wide as to hardly deserve the name duct. There is no distinction in histological character between the cells of the funnel, duct and brain; nor is there any indication either in the thickness of the walls or in any other feature of the boundaries between funnel, duct and brain. The ventral wall of the posterior part of the neural canal becomes thickened. This corresponds, as will be shown later, to the thick ventral wall of the "visceral portion" of the larval ascidian nervous system, *i. e.*, that portion which gives rise to the subneural gland. Later, the cells of the dorsal wall of the posterior part of the neural canal begin to multiply rapidly; some of them keeping their original arrangement as an epithelium bounding the canal; others, much more numerous, push up toward the ectoderm to form the dorsal portion of the adult ganglion. This proliferation of cells is greatest, for a time, at the most posterior part of the dorsal wall of the neural canal. The brain cavity has now a thick ventral wall, and a dorsal wall about twice as thick as the ventral. Fig. 4, Plate LI, shows one stage in the development of the ganglion of the solitary salpa. It will answer equally well for the chain individual.

At the time of the first appearance of the rudiment of the dorsal eye the three regions, funnel, duct and brain, are clearly distinguished, the duct being a small round tube connecting the lumen of the funnel with the cavity of the brain (Plate XLVII, Fig. 5). The description so far would answer almost equally well for either salpa, doliolum or pyrosoma, except that the latter two do not have the ventral wall of the neural canal thickened. Fig. 4, Plate XLVII, shows an oblique section through the duct at this stage. The lumen of the duct is cut in two places, at f near the brain where it is larger, at f_1 nearer the funnel where it is smaller and thinner-walled. The brain cavity at this time is much

flattened dorso-ventrally, forming a narrow slit separating the ventral third of the brain from the dorsal two-thirds.   As the cells of the dorsal wall of the neural canal push up to form the dorsal part of the ganglion they do not at once make a solid mass, but they leave between themselves fine, anastomosing, lucanar canals (Plate XLVII, Figs. 1 to 3, *l*), some of which open inward to the neural canal *f*, while others open outward to the cavity around the brain, *l'*.   These lacunæ are not mere chance spaces, but have a quite definite appearance and persist for some time.   In cross-section (Figs. 1 to 3, Plate XLVII) the lacunæ *l* are seen to be separated for the most part by single rows of cells resembling the liver cells between the bile ducts of higher vertebrates.   Soon the brain becomes solid, the lacunæ and also the central canal disappearing. When this change is nearly completed (Fig. 5, Plate XLVII), we see a condition almost exactly resembling that found in a nearly mature doliolum.   There is a slight antero-ventral process (shown in Fig. 5, Plate XLVII, in longitudinal section, and in Fig. 6, *v*, in cross-section) containing a remnant of the disappearing brain cavity, *f*.   This is still connected by a very fine canal with the wide lumen of the funnel.   The lumen of the canal does not show in the section drawn.   In the later development the brain cavity and the duct wholly disappears, and the lumen of the funnel is the only remnant of the neural canal.   The duct disintegrates as if pulled apart by the elongation of that region of the body, reminding one of the elongation of the duct in doliolum, by which the cells are pulled out into an interrupted pavement epithelium.   Neither in the half-developed chain individual nor in the adult can any trace of a duct opening into the funnel be found.

Certain other structures develop later that seem to bear the closest relation to the lateral communications between the subneural gland and the peribranchial chamber, described for phallusia mammillata.   At a time when the embryonic eye-disk has taken its perpendicular position (see the section on the development of the eyes of Cyclosalpa pinnata, chain form) the wall of the peribranchial chamber,[1] which up to this time has been close to the ventral surface of the brain (Figs. 1 to 3, Plate XLVII), begins to separate from the brain.   Two small areas remain

[1] Professor Brooks tells me that his study of the budding of salpa shows beyond question that the dorsal part of the pharynx is at this time still quite wide, so that the whole region beneath the ganglion and for a considerable distance laterally belongs to the branchial chamber.   The peribranchial chamber occupies anteriorly a very narrow region on each side of the body.

contiguous, however, with the brain, one situated at the right, the other at the left of the mid-ventral point of the ganglion (Fig. 1, Plate L). As the wall of the branchial chamber separates more and more from the surface of the ganglion, those portions of this wall which are adjacent to the two areas of adhesion are gradually drawn out into two tubes reaching from the ventral surface of the ganglion to the branchial chamber (Fig. 3, Plate L). The adult condition is reached by the considerable growth of these tubes, which become greatly coiled, and by the flattening out into a hollow disk of the portion of each tube contiguous with the brain. Figs. 4 to 6, Plate L, serial sections in a longitudinal, vertical plane, and Figs. 8 to 11, Plate L, serial cross-sections, show the adult condition. In the former series Fig. 4 is nearest the middle line of the body. In the latter series Fig. 8 represents the more posterior section. The structure of the cells lining the tubes and hollow disks (Plate L, Fig. 7) does not surely indicate their function. They are smaller than the cells of the brain, irregularly cubical in form, have well defined nuclei surrounded by finely granular protoplasm which, under very high magnification, shows minute vacuoles. These may or may not indicate secretory activity. The homologous cells in Salpa africana-maxima are almost surely glandular, so it is probable these cells in Cyclosalpa pinnata are more or less functional in secretion.

In close connection with the enlarged ends of the tubes just described there are four masses of cells, two on each side (Plate L, Figs. 4 to 6 and 10 and 11). One of these two masses, the more anterior (Figs. 4 and 11, b'), is composed of large cells with large nuclei, resembling closely the large ganglion cells that lie in the brain, in the zone of origin of the nerves (Fig. 1, n). The more posterior mass, b, is composed of small cells with small nuclei, having the appearance of the ordinary cells of the periphery of the brain. These masses of cells develop simultaneously with the lateral tubes (Plate L, Figs. 1 and 3) as outgrowths of the ventro-lateral areas of the brain. What present function they may have I am unable to say. Their homologies will be discussed later.

In the *solitary form* of *Cyclosalpa pinnata* the adult structures resemble minutely those described in the preceding pages for the chain form. (See Plate LI, Fig. 7.) The early stages of development, too, are the same. I have not traced the development for other species.

The structure of the subneural gland and the organs connected with it, in both *chain* and *solitary forms* of *Cyclosalpa Chamissonis*, is the same as in Cyclosalpa pinnata. In other species there is a certain

amount of variation. This is greater in the chain forms than in the solitary forms. *Salpa cylindrica, solitary form,* shows the same structure of the lateral tubes as Cyclosalpa pinnata, but there are no ventro-lateral outgrowths from the brain (Fig. 1, Plate LII). In the *chain form* the right tube is reduced to a mere funnel-shaped pit in the wall of the branchial chamber (Plate LII, Fig. 11, *d'*). The left is more developed, but does not extend to the surface of the brain. It arises from the wall of the branchial chamber at the level of the posterior surface of the brain and runs forward and toward the middle line, ending blindly beneath the center of the brain, without any enlargement comparable to the hollow disks found in Cyclosalpa pinnata (Plate LII, Figs. 10, 11 and 12, *d* and *do*). There are present in the chain form one pair of very slight ventro-lateral outgrowths from the brain, containing only the smaller kind of ganglion cells (Plate LII, Fig. 8, *b*). *Salpa scutigera-confederata, chain form,* shows the lateral tubes and disks typically developed, and a single pair of large-celled ventro-lateral outgrowths from the brain (Plate LV, Figs. 3, 4 and 5). I have not sectioned the solitary form. The *chain form* of *Salpa bicaudata* closely resembles that of Salpa scutigera-confederata in the structure of the subneural gland and adjacent organs (Plate LV, Figs. 8 and 9). In the *chain form* of *Salpa democratica-mucronata* there is no trace of lateral ducts or outgrowths from the brain. This is perhaps associated with the twisting of the ganglion described in the section on the eye. In the *solitary form,* on the other hand, the ducts are present in much the condition found in Cyclosalpa pinnata. Plate LV, Fig. 10, shows a longitudinal section cutting one of the hollow disks. There are no outgrowths from the brain. In the *solitary form* of *Salpa runcinata-fusiformis* the tubes and hollow disks are present and are unusually large (see Fig. 4, Plate LIII). Their epithelial lining is for the most part apparently lost, the walls of the tubes and of the greater part of the disks being formed seemingly only by the membrane continuous with the basal membrane of the wall of the branchial chamber.[1]

There is in the *chain form* of this species an antero-ventral outgrowth from the ganglion, shown in Fig. 9, Plate LIII: we shall see later that this is important. The *chain form* of *Salpa costata-Tillenii* shows a different condition of these organs. The wall of the branchial

---

[1] It is probable that those portions of the tubes which appear in section to have no epithelial lining have in reality a lining of pavement epithelium not visible in section.

chamber remains attached to the whole ventral surface of the brain (see Fig. 10, Plate LIV), forming a single large pit opening by a wide mouth to the peribranchial chamber. The cells of that portion of this pit which is attached to the ganglion show the structure typical for the cells of the hollow disks. In this species alone is the subneural gland an unpaired structure. The *chain form* of *Salpa africana-maxima* shows the greatest development of the tubes and hollow disks. The latter are very large (see Fig. 9, Plate LIV) and are lined by an epithelium composed for the most part of a single layer of cubical cells whose outer ends are uneven, whose protoplasm is granular and does not take up hæmatoxylin stain, and which have an appearance as if glandular in function. On the dorsal sides of the disks the epithelial cells are greatly increased in number, forming an irregular mass that half fills the lumen of the disk. In all species the epithelium of the dorsal wall of the disk is more developed, and in some species (Salpa democratica-mucronata) only this portion has any epithelial lining that can be distinguished in section. Probably there is a thin pavement epithelium over the other portions. The ducts, one on each side as in other species, are unusually large. They are lined by an epithelium of columnar cells whose outer ends are very irregular. I believe we have in this species a gland which is functional. The epithelium of both disks and ducts has the characteristic, granular, vacuolated protoplasm, and seems to be degenerating to form the secretion. The fact that the hollow disks form the dorso-lateral walls of the great dorsal blood sinus, which gives many branches to the dorsal lamina or gill, renders it easily credible that these structures should function as a gland. If this gland be functional in this species, it is the only case I have observed where the secretion could be considerable.

The nervous system of Salpa africana-maxima shows another feature which is of the keenest interest. We have seen that in the chain form of Salpa runcinata-fusiformis there is an antero-ventral outgrowth from the ganglion. This resembles the "wart-like," antero-ventral process from the ganglion of doliolum. In Salpa africana-maxima there is a similar antero-ventral outgrowth from the ganglion (Plate LIV, Fig. 3), and *this is produced anteriorly* as a rod of cells which runs forward beneath the eye, soon meeting the pharynx wall. It grows smaller, but still can be traced, close pressed to the basement membrane of the pharynx wall, running on toward the ciliated funnel. It finally fuses with the pharynx wall, but can be traced for a comparatively long

distance further as a very fine canal within the basement membrane (see Plate LIV, Figs. 3–8). Unfortunately, the only two specimens of the chain Salpa africana-maxima I had for study were injured, having been brought up from some depth on thermometer lines and having been in this way badly torn. In one of these the ganglion was missing; in the other the ciliated funnel; so I have been unable to certainly establish the relation of this rod of cells to the ciliated funnel. I am convinced, however, that in this rod of cells we have the homologue of the tube which in doliolum connects the ciliated funnel with the wart-like antero-ventral process from the ganglion. If this be so, we have in Salpa africana-maxima a condition that is found in doliolum and in the young chain Cyclosalpa pinnata.[1] The nervous system of Salpa africana-maxima is then, in this respect, more primitive than that of any other adult salpa I have studied.

What is the relation of the ganglion, the subneural gland and its accessory structures in salpa to the organs described in the first part of this section for doliolum, pyrosoma and ascidians? The ganglion of salpa has been regarded by Salensky [14] and others as homologous with the whole central nervous system of the ascidian larva. He has described three slight enlargements of the neural canal of the embryonic solitary salpa which he says correspond to the three primary vesicles of the vertebrate brain and to the three regions of the larval ascidian neural canal—the sense vesicle, the visceral portion and the caudal portion respectively. A careful comparison of the conditions in the different ascidians and in pyrosoma, doliolum and salpa, seems to me, on the contrary, to indicate that the ganglion of salpa is homologous with only the visceral portion of the larval ascidian nervous system. In favor of this homology there are the following points:

1. It is only in the visceral portion of the larval ascidian nervous system that the ventral wall of the neural canal is thickened. Only that portion of the embryonic salpa's neural canal which has a thickened ventral wall persists to form the adult ganglion.

2. The adult ascidian ganglion is formed from the dorsal cells of the visceral portion of the larval nervous system. The main portion (dorsal two-thirds or three-fourths) of the adult salpa ganglion is derived from

[1] A similar tube is found in the embryo of the solitary form, connecting the ganglion with the ciliated funnel, Plate LI, Fig. 4.

the dorsal cells of that portion of the embryonic nervous system which has a thickened ventral wall. The remainder is formed from this thickened portion of the ventral wall of the neural canal. The dorsal part of the ganglion would seem, then, to be certainly homologous with the ganglion of ascidians and pyrosoma; it being a structure of secondary formation in each and having similar origin. In doliolum, if we had full data for judging, it is probable a similar origin would be found for the dorsal part of the adult ganglion. The almost complete resemblance between the immature salpa's nervous system and that of the nearly mature doliolum argues strongly in favor of this.

3. The relation of the dorsal and ventral portions of the immature salpa's nervous system to the duct that opens into the funnel is much the same as the relation of the ganglion and subneural gland to the duct of that gland in the older larvæ of many ascidians, e. g., amarœcium. In this connection the lacunæ in the immature salpa ganglion, opening into the central canal of the nervous system and, on the other hand, the connection between the larval ascidian ganglion and the duct are suggestive.

4. The caudal portion of the larval ascidian nervous system degenerates. In salpa there is an absence of any caudal portion of the nervous system, correlated with the absence of any normally developed tail.

5. The sense vesicle of the larval ascidian is not present in any recognizable form in either salpa, doliolum, or pyrosoma at any known stage of their development. It seems to be a special larval character secondarily acquired by the ascidians. (See also the last paragraph of Appendix I.)

6. The ventral wall of the visceral portion of the ascidian tadpole's nervous system proliferates cells to form the body of the subneural gland; the whole ventral wall being apparently thus used up. In salpa most of the thick ventral wall persists to form the ventral third of the ganglion, but certain cells push out ventrally toward the hollow disks of the subneural gland, recalling the similar, though more extensive, migration of "nerve-gland" cells in the ascidian larva.

If the ganglia of salpa and the ascidians be homologous, as seems so evident, the three so-called vesicles described by Salensky in the immature ganglion of Salpa scutigera-confederata lose the importance assigned them by Salensky. They are then to be regarded as secondary, and having no morphological connection with the three primary vesicles of the vertebrate brain, or with the three regions of the neural canal in the ascidian tadpole.

If then we homologize the dorsal part of the ganglion in salpa with the adult ascidian ganglion, and the ventral third or quarter of the salpa ganglion (the original thickened portion of the ventral wall of the neural canal) *with the thick ventral wall of the visceral portion of the larval ascidian nervous system, we are justified in saying that the ascidian gland,* which is formed from the thick ventral wall of the visceral portion of the larval nervous system, *is represented in salpa by the ventral portion of the ganglion.* Of course this never functions in salpa as a gland. The homology is strengthened by the presence of the lateral tubes in salpa, which connect the two hollow disks upon the ventral surface of the ganglion with the branchial chamber. These seem to be very closely related to the communications between the subneural gland and the peribranchial chamber, found in Phallusia mammillata. The comparison is made more perfect by Herdman's description [4] of two individuals of Phallusia mammillata in which the duct of the subneural gland leading to the ciliated funnel had atrophied.

The bearing of this homology upon the relation of the eye of salpa to the larval ascidian eye and the pineal eye of vertebrates is evident at a glance. *Salpa eye is a new structure wholly unrelated to the ascidian eye.* It develops from cells which have their origin in an entirely different region of the nervous system. *It cannot be homologized with either of the epiphyses of the vertebrate brain,* for it does not arise directly from the central nervous system as do the latter, but arises from a secondarily acquired, modified portion of the nervous system, not represented in the vertebrate brain. For the same reason the pigment spots in the ventral part of the ganglion of pyrosoma cannot be homologized with the larval ascidian eye or the pineal eye of vertebrates, as Seeliger [17, p. 582] claims, but they are more probably related to the posterior or posteroventral eyes of such species as Cyclosalpa pinnata or Salpa costata-Tillesii.

Does the condition of the nervous system and subneural gland of salpa furnish any evidence as to the *relationship of salpa to the other tunicates?* I have shown the almost complete resemblance between the nervous systems of salpa and doliolum at a certain stage of their development. *The nervous system of salpa passes through a doliolum stage.* This would be evidence then, so far as it goes, that doliolum and salpa had a common ancestor and that doliolum stands the nearer to that ancestor. The nervous system of pyrosoma, though a little more removed from that of salpa, still very closely resembles it. The two most important differ-

ences are the persistence of the neural canal in the adult pyrosoma[1] and the very slight degree of thickening of its ventral wall. The latter of these is a point of difference from all other tunicates and is probably to be regarded as secondary. In comparing the nervous systems of the ascidians, pyrosoma, doliolum and salpa, we find the latter three much more alike than either of them is to the ascidians. Of the latter three, salpa appears to present the most resemblance to the ascidians, because of the presence in these two alone of a subneural gland with paired lateral ducts; but this point is not one from which alone relative degrees of relationship can safely be deduced, for the same structures may once have been present in pyrosoma and doliolum, though now they are lost in these genera. The ease with which they may be lost is shown by the fact that in the chain Salpa democratica-mucronata they have disappeared. The presence of these structures in ascidians and salpa does strongly indicate that the common ancestor of the two had a subneural gland with one or more pairs of lateral ducts. Emphasis is given to this view by the fact that in Salpa africana-maxima, chain form, whose nervous system shows the most primitive character, we have the greatest development of the gland.

The lateral ducts of the subneural gland in Phallusia mammillata connect with the peribranchial chamber, according to Van Beneden and Julin, while in salpa the lateral ducts connect with the pharynx or branchial chamber. This difference at first sight seems to preclude the homology of these organs in the two cases. But still, after careful study, it seems to me the structures may be truly homologous for several reasons. They have a decided superficial resemblance. They are located in the same region with reference to the ganglion. They have the same function; at least in both cases they are gland ducts. It is furthermore easy to conceive a shifting of position by which the lateral ducts, originally opening to the peribranchial chamber, came to open to the branchial chamber. The peribranchial and branchial chambers in salpa are later-

---

[1] Salensky [14] regards the canal opening into the funnel of the adult pyrosoma as a new structure and not as a remnant of the canal of the central nervous system. Whether it be a new structure or the original canal reopened, there is at any rate a closing and at least partial degeneration of the canal of the central nervous system previous to the appearance of the definitive funnel. This degeneration is very probably homologous to the similar degeneration of the anterior portion of the canal of the central nervous system observed in salpa and doliolum (and probably also to the degeneration of the duct leading from the gland to the funnel seen in some individuals of the ascidian, Phallusia mammillata).

ally separated by no distinguishable boundary. I could not from my own study of the adult salpa and the later stages of development ascertain whether the lateral ducts opened to the pharynx or to the lateral, anterior prolongations of the peribranchial chamber. Dr. Brooks, however, tells me that at this time (the time when the lateral ducts appear) the anterior portion of the peribranchial chamber is limited to two very narrow lateral areas. The ducts, on the other hand, are not far from the median line. The whole formation of the peribranchial chamber in salpa is so much modified that it is but a slight additional change to suppose that the openings of the lateral ducts of the subneural gland shifted their position a little more toward the median line and into the region of the pharynx, and that later, their development being somewhat accelerated, they came to be formed before the anterior horns of the peribranchial chamber develop. The greatest degree of concentration of the lateral ducts toward the mid-dorsal line is seen in Salpa costata-Tillesii, in which the whole gland is but a widely open pit on the mid-dorsal line of the pharynx. It seems probable then that we should regard the connection of this gland in salpa with the branchial instead of the peribranchial chamber as a secondary modification consequent upon the obliteration of the lateral walls separating the two regions. Assuming for the present the truth of this hypothesis, and therefore the homology of the subneural gland in salpa and Phallusia mammillata, some interesting comparisons may be made.

The fact that in all but one of the eleven species of salpa studied this gland is paired is suggestive, but in view of the fact that in Phallusia mammillata[1] and in one species of salpa (Salpa costata-Tillesii) it is unpaired, it is doubtful whether in the common ancestor of the two the gland was a paired structure. The transition from a paired to an unpaired structure, or *vice versa*, would be very easy. This is seen when we consider the mode of its development in salpa.

Phallusia mammillata has a subneural gland of twofold origin; a part coming from the central nervous system; a part from the wall of the peribranchial chamber. Most of the ascidians have a simple gland formed solely from the nervous system. Salpa has a subneural gland formed almost solely from the wall of the branchial, originally peribranchial (?) chamber. It is not probable that the earliest ascidians had a gland of twofold origin. The question then confronts us, which sort of subneural gland is the more primitive? The natural though not

[1] In Phallusia mammillata the ducts, though not the gland itself, are paired.

undoubted answer would be "that found in the most widely separated species." This would indicate that the gland arising from the peribranchial chamber is the more primitive, since it is found in one of the ascidians (Phallusia mammillata) and also in salpa. Prof. Herdman, in his paper already referred to and in other more recent papers [5], makes an interesting suggestion that "if the hypophysis cerebri was, as E. van Beneden and Julin think, a renal organ in the anterior part of the body of the primitive chordata, its ducts—supposing it to be formed of several pairs of nephridia—would originally open on the sides of the body, and might have then become implicated in the sinking-in of the epiblast to form the atrial involutions, and so would come to open into the peribranchial cavity (a condition found in some ascidians)." He says, "I would regard then the connection between the duct, or one of the ducts, of the subneural gland and the dorsal tubercle as being secondary." According to this hypothesis, Phallusia mammillata shows a transitional phase; most ascidians have passed beyond and have lost the peribranchial portion of the gland. Salpa would then have reverted to the condition found in one of the more primitive ascidians having a well developed peribranchial gland. It is probable, however, that the presence in salpa of the ventro-lateral outgrowths from the brain toward the hollow disks (of the peribranchial gland) indicates the partial retention of a process similar to that by which the subneural gland of ascidians is formed at the expense of the thick ventral wall of the visceral portion of the larval neural canal. The innervation of the large-celled pair of these outgrowths (Plate L, Fig. 4, b') may indicate that they were formerly, if not at present, functional as ganglia in connection with a well developed gland which arose partly from the wall of the peribranchial chamber and partly from the nervous system.

On the other hand, if a gland of neural origin be the more primitive, Phallusia mammillata would still represent the transition from this condition to the gland derived from the wall of the peribranchial chamber. I incline to the former hypothesis; but, be this as it may, salpa has retained or reverted to the condition found in a transitional form like Phallusia mammillata, since it shows traces of a gland of double origin. Molgula ampulloides has two well developed lateral chambers, one on each side of the gland, which open through the duct of the gland into the pharynx by way of the ciliated funnel. These are of interest in this connection, for they may correspond to the hollow disks of salpa's gland. Molgula ampulloides would then represent a second transitional form

between the condition in Phallusia mammillata and that of other ascidians, since it has no lateral ducts. These comparisons are all hypothetical, with little direct evidence in their favor, yet they are well worth considering.

What is the significance of the lacunæ in the dorsal part of the ganglion of salpa? They have too definite a structure to be regarded as mere chance spaces between the proliferating cells. They seem to me to indicate that the former function of the hypophysis was to aerate the brain or to carry off the waste products of its metabolism, or perhaps both. In many of the ascidians we find that at one point in the dorsal wall of the duct of the gland the epithelial lining of the duct is wanting, and the cells of the ganglion reach the lumen of the duct. This condition found in so many simple ascidians must have some meaning. It seems to bear a relation to the lacunæ in salpa's ganglion and to confirm my interpretation of the latter. We have seen that the lacunæ connect with the lumen of the neural tube, the homologue of the duct of the ascidian gland.

The great variation in the subneural gland as to its presence or absence in the solitary or chain forms of different species, and especially the variation in the ventro-lateral outgrowths from the ganglion, would indicate that these structures are now of little functional value.

The fact that in the salpas we have present well developed eyes which yet cannot be homologized with the larval ascidian eye is suggestive in regard to a question which Professor Brooks discusses in the main portion of this memoir, namely, the question of salpa's descent from an attached ascidian-like ancestor, or from a free swimming form like the ascidian larva. If, accepting the latter hypothesis, there had been no break in the free swimming manner of life from the ascidian tadpole to its descendant salpa, there would never have been a time in its phylogenetic development when an eye would not have been needed, and so we would naturally expect to find salpa in possession of an eye directly traceable to the ascidian larval eye. This, however, is not the fact. The eye of salpa is situated in a wholly different region of the brain from that in which the larval ascidian eye is found. It would seem, then, more natural to suppose that salpa is descended from some attached form, which, having given up its free swimming life, would have little use for an optic organ and would therefore have lost the eye which it originally possessed. Salpa, on resuming a free swimming habit, would need a visual organ; which, in fact, we find. The

attached form, which the second hypothesis regards as salpa's progenitor, would probably have lost the greatly developed sense-vesicle which we see in the ascidian larva, so that the free swimming primitive salpa would have to develop an eye in a new region of the brain.   At the same time it would be most natural that the new eye should develop about the same structure as is found in the eye possessed by its remote free swimming ancestor (the ascidian tadpole).   This hypothesis, then, explains perfectly the fact that the eye of salpa, while having essentially the same structure as the larval ascidian eye, is found in a different part of the brain and that the two are in no way homologous.

The secondary manner of formation of the ganglion itself in pyrosoma, doliolum and salpa would argue that they are descended from some common form which derived its ganglion from a secondary modification of the central nervous system, *i. e.* from an ascidian-like, rather than a tadpole-like form.

APPENDIX I.

On page 349 I mentioned the conflict between Kowalevsky and Van Beneden and Julin, in regard to the presence in the ascidians of an anterior opening of the neural canal into the peribranchial chamber. Kowalevsky's statement that such a communication is present has lately been confirmed by Willey [21], working independently upon different species of ascidians. There is, then, close agreement in this respect between the four groups of tunicates.

Willey says: "As stated by Kowalevsky, the neuroporus of the ascidian embryo closes up at an early stage of development, and the nervous system then consists of a perfectly closed tube with a dilated anterior extremity lying below the epidermis. Soon after the invagination of the stomodæum and the subsequent perforation of the mouth, the nerve tube acquires secondarily an opening into the stomodæum . . ." This statement is of interest in connection with Salensky's description of a similar closing of the neuropore in pyrosoma, and a subsequent formation of a new connection between the gut and the lumen of the neural tube. It is probably true, as Willey seems to imply for the ascidians, that in both ascidians and pyrosoma this secondarily established communication is but the reopening of the original neuropore.

Willey has shown also that the first rudiment of the definitive ganglion in Cionia intestinalis appears as a slight thickening of the dorsal wall of the neural tube. This corresponds exactly to Van Beneden's and Julin's description and to what I have described for salpa, making still more indubitable the already accepted homology between the definitive ganglia in salpa and ascidians, and establishing to my mind conclusively the homology between the visceral portion of the neural tube of the ascidian tadpole and that portion of salpa's neural tube from which the ganglion is formed.

APPENDIX II.

While my paper was in press, Professor Bütschli [2] published a short note upon the eye of salpa. I discussed briefly in the Zoologischer Anzeiger [11] the theoretical conclusions of his paper. I wish here also to review it. Lest my criticisms should seem severe, let it be borne in mind that Professor Bütschli expressly says that when he wrote his brief note he did not have access to the material upon which he had worked, or to his preparations, notes, or figures, but that he wrote entirely from memory. Moreover, his work upon this subject was confessedly incomplete, so that his suggestions were not the expression of his judgment carefully matured after thorough investigation, but were rather the conclusions toward which the work seemed to be leading him. The paper is written, however, with Professor Bütschli's characteristic clearness and suggestiveness, and his conclusions are so plausible that they deserve careful examination.

First, let me call attention to one anatomical point. Professor Bütschli says that the retina is composed of two sorts of cells, the visual cells and supporting cells ("Stützzellen"), and that the latter by their anastomosing processes form a network in whose interspaces the visual cells are held. As I have shown, this network is not a network of cell processes, but is really the darkly staining cell walls of the more deeply lying portion of the visual cells. In some cases (see Fig. 14, Plate LII) the visual cells wholly, or in part, lose their columnar form and regular arrangement in a single layer. The arrangement may even become wholly irregular (see Fig. 10, Plate LIV), while at the same time they assume very irregular shapes. They still retain, however, the tendency to thicken their cell walls. The appearance seen in stained sections, which resembles an irregular network of cells, is not a network of branched cells, but is formed by the thickened walls of the irregularly shaped, probably degenerate cells, which once functioned and perhaps still function as visual cells. Because of this tendency to thicken their cell walls, I have throughout my paper called them rod cells. They are undoubtedly closely related structurally and physiologically to the rod

cells.in vertebrate eyes, which always show a similar thickening of their cell walls.

Professor Butschli says the horseshoe-shaped eye is secondary, being derived from a "hügelartig" eye, of which he gives a schematic figure. His explanation of how the horseshoe-shaped eye is derived from the "hügelartig" form is so very neat that it predisposes one in its favor. Let us, however, examine it. There is no eye among the solitary salpæ to which his description ("hügelartig") could at all apply. Unless he studied some very aberrant species which I have not seen, he must refer to such an eye as is found in the chain form of Salpa runcinata-fusiformis or some of its nearest allies. In this group the larger dorsal eye is either spindle-shaped or globular and answers roughly to his description. He says: "Die Hauptmasse dieses Hügels [the eye] wird von einer lichtempfindlichen Retina gebildet, deren Zellen der freien Wölbung des Hügels entsprechend, etwas radiär zu der Oberfläche gestellt sind. An seiner basalen Peripherie wird dieses hügelartige Auge von einem ringförmigen Gürtel von Pigmentzellen umzogen ... Das geschilderte einfache Auge empfängt seiner Nervenfasern direct von unten aus dem Gehirn." The structure of this eye is not so simple as this description says. In none of the eleven species I have studied do the rod cells all have the same arrangement. In every case, as I have shown, where the eye is globular or spindle-shaped, the rod cells of the posterior portion of the eye have their innervated ends distal from the ganglion, while in the anterior part of the eye this arrangement is reversed. The pigment cells also show an arrangement different in these two portions of the eye, and do not form what can be called a simple girdle at its base. The rod cells, instead of receiving their innervating fibers direct from the ganglion, receive them from a large optic nerve which passes up over the *dorsal* surface of the posterior part of the eye and then through to the ventral side of the anterior portion. These three mistaken characters in his description are three of the four points he emphasizes to show the primitive character of this form of eye. The fourth point is its simple shape.

Again, we would not naturally expect to find the primitive type of salpa eye in the chain form of any species, since the chain individuals are asexual,[1] and are derived from the solitary, sexual form by a peculiar kind of budding. The chain individuals, then, being secondary, the pre-

[1] Professor Brooks has often emphasized this asexuality. In the present work, of which my paper forms a part, he shows it with perfect conclusiveness.

sumption is strong that their form of eye would be secondary. Emphasis is given to this view by the fact that the same type of eye (horseshoe-shaped) is found in the solitary form of each species, while the chain form of each species has a type of eye peculiar to itself.

The secondary nature of the "hügelartig" eye, and of all other sorts found in the chain salpæ, and the primitive character of the horseshoe-shaped eye, is conclusively shown by the fact that the eye of the chain individual passes through an ontogenetic stage when in form, though, of course, not in histological character, it closely resembles the horse-shoe-shaped eye of the solitary salpa.

Professor Butschli's suggestion as to the probable primitive mode of origin of the vertebrate lateral eyes is based upon the secondary nature of the horseshoe-shaped eye and its manner of development from the "hügelartig" eye. If the latter be overthrown, the former is without foundation.

The most important point, however, is not that for certain special reasons founded upon the comparative anatomy and ontogeny of the salpa eye, the relations suggested between this eye and the vertebrate lateral eyes, are mistaken. It is fundamentally erroneous to homologize the eye of salpa with the eye of any other chordate. The salpa eye is developed from a highly modified portion of the nervous system, which is not represented in the vertebrate brain. This portion, the ganglion, is derived from the posterior part of the neural canal. The eyes of all other chordates are developed from the walls of the *first primary vesicle* or its homologue. For further elaboration of this point see page 355 to 357 of this paper, and also a brief discussion in my paper, "The eyes and subneural gland of salpa," in the Zoologischer Anzeiger [11].

APPENDIX III.

Since my paper was in press, Dr. E. Göppert has published an excellent paper [3] upon the eye of salpa. The author had evidently not seen my preliminary abstract, so it is the more gratifying to see how completely, so far as he goes, he confirms my results. He gives a good account of the anatomy of the eye of both the chain and solitary form of Salpa Africana-maxima, Salpa runcinata-fusiformis, Salpa (Cyclosalpa) pinnata, Salpa democratica-mucronata and Salpa scutigera-confederata. His description of the minute internal anatomy of the rod cells, especially as regards the so-called phæospheres, is more elaborate and therefore better than my own.

There are but few points upon which I must take exception to his statements, and few others upon which I need comment. There is, however, one fundamental disagreement between our observations, namely, in regard to the innervation of the rod cells. I may state at once that if I had not had opportunity to study the development of the eye I would have been unable to speak with any certainty of the innervation of one portion of it, that portion upon which our observations disagree. In his description of the larger dorsal eye of the chain individuals of Salpa Africana-maxima, Salpa runcinata-fusiformis and Salpa (Cyclosalpa) pinnata, Göppert says that in the posterior portion of the eye the rod cells receive the innervating fibers into their thin-walled ends, while in the anterior portion they receive the innervating fibers into their thick-walled ends. Reference to my Figs. 7, 8 and 9, Plate XLVIII, will show that this is a mistaken statement. Fig. 7, *on*, shows the large optic nerve above one of the posterior limbs of the eye (*e'*), in which region it directly innervates the thin-walled ends of the rod cells which lie just beneath it. Fig. 8 shows the innervation of the third region of the eye (*e'''*). That the rod cells of this region receive their innervating fibers into their thin-walled ends is more clearly shown in Fig. 7, Plate XLIX. Fig. 9, Plate XLVIII, shows another bundle of fibers from the optic nerve, *on''*, passing down

between the two posterior limbs of the eye (*e'*) to innervate the ventrally placed thin-walled ends of the rod cells of the second, the anterior portion of the eye (*e''*). The rod cells, then, in all regions of the eye of the chain Cyclosalpa pinnata are innervated at their thin-walled ends, the ends distal from the pigment layer. Such a fundamental difference in the innervation of the different regions of the eye as Göppert describes would be utterly inexplicable. It is but fair to say that in the adult eye, which alone Göppert studied, the fibers of the optic nerve are more closely pressed to the rod cells than in the immature individuals. It is therefore a matter of great difficulty to determine the innervation from the study of serial sections of the adult. The minute fibers innervating the rod cells of the anterior portion of the eye cannot be traced in section. The optic nerve appears to stop at the junction of the posterior limbs of the eye with the anterior curved portion. That this appearance is really deceptive is shown by my sections of the immature individuals. But even in the absence of the actual proof I have brought forward we would be justified in believing that the innervation in all regions is uniform, unless there were much more definite observations to the contrary than any Dr. Göppert has made.

In the immature chain Cyclosalpa pinnata it is plainly seen that the rod cells in all regions are innervated from their thin-walled ends. In the adult chain Cyclosalpa pinnata the innervation of the anterior region of the eye cannot be made out. In other chain-salpæ of the pinnata group the innervation of the anterior region of the dorsal eye can no more be made out in the adult than in the adult Cyclosalpa pinnata. The innervation of the posterior region is plainly seen to be the same as in the latter species. The anterior portion of the dorsal eye is clearly homologous in all these species. In consideration of these facts we must believe that the whole innervation in all the species is as in Cyclosalpa pinnata. In the chain Salpa democratica-mucronata, which I have shown belongs to the pinnata group since the dorsal eye is here reversed, Göppert himself says the rod cells of all regions are innervated from their thin-walled ends. In the more primitive group, including Salpa scutigera-confederata and Salpa bicaudata, my figures show as clearly as can be shown from sections of the adult eye that the manner of innervation is the same. The innervation of the anterior horns of the horseshoe-shaped eye of the solitary form is also difficult to make out, but since the rod cells of the posterior curved portion are plainly seen to receive the fibers into their thin-walled ends, there is no room for doubt that the

rod cells of the anterior horns also are innervated at their thin-walled ends.[1]

Göppert's description of the histology of the eye of the solitary Salpa Africana-maxima, which I was unable to study in section, agrees very closely with my description of the histological condition of the eye of the solitary Salpa runcinata-fusiformis; thus confirming my view that these two species are very closely related.

Of the connection between the rod cells and intermediate cells in the larger eye of the chain Salpa Africana-maxima, Göppert says: "Ich glaube mit aller sicherheit behaupten zu können, dass jede der grossen Zellen [rod cells] durch ihren, die mittlere Retinaschicht durchziehenden Fortsatz mit einer dieser kleinen Zellen [intermediate cells] verbunden ist"; thus agreeing with the opinion I have stated in my account of the eye of Salpa runcinata-fusiformis. His figure illustrating the point is, however, more diagrammatic than any of my sections.

That Göppert fails to see the homology between the regions of the eye of the chain Salpa scutigera-confederata with those of the eye of any other species is only natural, since he did not clearly grasp the idea of the reversal of the eye in most species and the consequent change in the course of the optic nerve. It is interesting to note that he does suggest, though not with much confidence, that there has perhaps been a forward bending of the eye in the chain individuals, a thing which I had already shown to be a fact [12].

Leuckart's statement, which Göppert quotes apparently with approval, that the pigment layer is always superficial, immediately under the epidermis, is clearly shown to be inaccurate by my Figs. 7 and 8, Plate LI; 2, Plate LII; 10, *px*, Plate LIV.

Dr. Göppert emphasizes the statement that at least in the dorsal eye of salpa the rod cells of the different regions receive light from but one direction, thus enabling the animal to perceive direction. Whether the facts of the anatomy lend support to this view I am unable to decide. It is not improbable. I am much less able to accept as true the author's assumption that the masses of rod-like cells which occur in the ganglion and are not associated with pigment, are functional optic

[1] Göppert describes in Cyclosalpa pinnata, solitary form, a connection of the nerve fibers with the ends of the rod cells near the pigment layer, while in the anterior part of the retina of the chain Salpa Africana-maxima he says the nerve fibers connect with the cells of the intermediate layer. His whole account of the innervation is anomalous.

organs. The author's casually stated opinion that the lateral chambers of the subneural gland, which he observed, are organs of hearing, is but a sample of the far too many statements without study made by students of the tunicates, which have only added to the confusion.

Göppert calls attention to one point that had entirely escaped me, namely, that there is a definite relation in most species between the position of the chain-salpa in the chain and the position of the eye. He points out that in individuals from the right side of the chain the long axis of the eye is directed obliquely toward the right, while in indi_viduals from the left side of the chain the eye points toward the left. This gives importance to a slight though constant peculiarity I have observed in the otherwise symmetrical eye of the chain Cyclosalpa pinnata, which serves to determine the relation of the animal to the stolon. In this species the individuals stand with both their antero-posterior and their dorso-ventral axes at right angles to the stolon. In the large dorsal eye of each individual, on the side distal from the former attached end of the stolon, there is a small unpigmented spot in the midst of the pigment area of the second region of the eye. (See Fig. 10, Plate LVII, *up.*)

LIST OF PAPERS REFERRED TO IN PART IV.

[1]. BENEDEN, ED. VAN, et CH. JULIN. Le systeme nerveaux central des Ascidies adultes et ses rapports avec celui des larves urodeles. Bull. de l'Acad. Roy. de Belgique, 3e Serie, 8, 1884.

[2]. BÜTSCHLI, O. Einige Bemerkungen über die Augen der Salpen. Zool. Anz., Sept. 1892, No. 401.

[3]. GÖPPERT, E. Untersuchungen über das Sehorgan der Salpen. Morph. Jahrbuch, Bd. 19, Heft 3.

[4]. HERDMAN, W. A. On the Homology of the Neural Gland in the Tunicata with the Hypophysis cerebri. Abstract Proc. Roy. Soc. Edinb., Vol. XII, 1882–83, p. 145; also in Nature, Vol. 28, p. 284–86.

[5]. HERDMAN, W. A. Recent Discoveries in connection with the Pineal and Pituitary Bodies of the Brain. Proc. Liverpool Biol. Soc., Vol. I, p. 18.

[6]. HJORT, JOHAN. Zum Entwicklungscyclus der zusammengesetzten Ascidien. Zool. Anz., Sept. 1892, No. 400.

[7]. JOLIET, L. Sur le developpement du ganglion et du " sac cilie " dans le bourgeon du Pyrosome. Compt. rend. de l'Acad. des Sciences, Paris, Tome 94, p. 988.

[8]. JULIN, CH. Recherches sur l'organization des Ascidies simples. Sur l'hypophyse et quelques organes qui s'y rattachent chez Ascidia compressa et Phallusia mammillata. Arch. de Biol., Tome II, 1881.

[9]. KOWALEVSKY, A. Weitere Studien über die einfachen Ascidien. Arch. f. mik. Anat., Bd. VII, 1871, p. 101.

[10]. LAHILLE, FERNARD. Recherches sur les Tuniciers des cotes de France. Toulouse, 1890.

[11]. METCALF, M. M. On the Eyes, Subneural Gland and Central Nervous System in Salpa. Zool. Anz., No. 409, 1893.

[12]. METCALF, M. M. The Anatomy and Development of the Eyes and Subneural Gland in Salpa. A Preliminary Note. Johns Hopkins Univ. Circ., Vol. XI, No. 97, April, 1892.

[13]. OKA, ASAJIRO. Über die Knospung der Botrylliden. Zeit. f. wiss. Zool., Bd. 54, Heft 3, October, 1892.

[14]. SALENSKY, W. Beiträge zur Embryonale-entwicklung der Pyrosomen. Zool. Jahrbücher (Spengel), Bd. 5, Heft 1, October, 1891.

[15]. SALENSKY, W. Neue Untersuchungen über die embryonale Entwicklung der Salpen. Mittheil. aus der zool. Stat. zu Neapel, Bd. 4, 1883.

[16]. SEELIGER, OSW. Zur Entwicklungsgeschichte der Pyrosomen. Jena. Zeitschrift f. Naturwiss., Bd. 23, 1889.

[17]. SEELIGER, OSW. Die Entwicklungsgeschichte der socialen Ascidien. Two parts. Jena. Zeitschrift f. Naturwiss., Bd. 18, 1885.

[18]. SHELDON, LILIAN. Note on the ciliated Pit of Ascidians and its Relation to the Nerve-ganglion and so-called Hypophysial Gland; and an Account of the Anatomy of Cynthia rustica (?). Q. J. M. S., Vol. 28, 1888.

[19]. TRAUSTEDT, M. P. A. Spolia Atlantica, Bidrag til Kundskab om Salperne. Vidensk. Selsk. Skr., 6, Raekke, Naturvidenskabelig og Mathematisk, Afd. II, 8.

[20]. ULJANIN, B. Die Arten der Gattung Doliolum im Golfe von Neapel und den angrenzenden Meeresabschnitten. Eine Monographie. Leipzig, 1884. [Fauna und Flora des Golfes von Neapel.]

[21]. WILLEY, ARTHUR. On the Development of the Hypophysis in the Ascidians. Zool. Anz., Sept. 1892, No. 400.

SALPA

W. K. BROOKS

PART FIVE.

EXPLANATION OF THE PLATES.

EXPLANATION OF PLATES I TO XLVI.

Unless it is otherwise stated, the colors in the plates designate the following structures: Red, the endoderm and organs which are endodermål in origin, and also, in the figures of eggs and embryos, the epithelial capsule and the structures which are derived from it; blue, the follicle, and the structures which are derived from it; yellow, the body cavity, the heart, and the blood spaces; green, the perithoracic tubes, and cloaca or atrium; purple, the endoderm and its derivatives; orange, the eggs and the blastomeres.

In Plates IX, X, XI and XII, the perithoracic tubes and the median atrium or cloaca are colored red, while they are colored green in all the other plates.

REFERENCE LETTERS.

a, Ectoderm.

a', Ectodermal layer of stolon.

a'', Reflected layer of ectoderm around the root of the stolon.

a''', Ectodermal blastomeres of embryo.

b, Endoderm.

b', Epithelial capsule of embryo.

c, Pharynx.

cb, Peripharyngeal band.

cg, Dorsal tubercle.

d, Endostyle.

d 1, Right half of endostyle.

d 2, Left half of endostyle.

d', Endodermal tube of stolon.

d'', Wall of endodermal tube of stolon.

e, Heart.

em, Embryo.

end, Endostyle.

f, Pericardium.

g, Right perithoracic tube of the stolon and of the aggregated salpa.

g', Perithoracic tubes of embryo.

g'', Spiracle or external opening of perithoracic tube.

g''', Median atrium or cloaca.

*g*IV, Gill-slits.

*g*V, Atrial or cloacal aperture.

h, Left perithoracic tube of stolon and of aggregated salpa.

i, Lower blood tube of stolon.

int, Intestine.

j, Upper blood tube of stolon.

k, Eleoblast.

l, Nerve tube of stolon.

m, Follicle.

n, Ovary or eggs. In Plate III, Fig. 3, the nucleus.

o, The dorsal lamella or "gill."

p, Intestine.

p', Stomach.

P'', Anus.

pl, Placenta.

ph, In Plate IV, œsophagus.

r, Peripharyngeal bands.

s, Ganglion.

st, Stolon.

t, Subneural gland.

u, Muscles.

v, Cellulose mantle.

w, Oral muscles.

x, Fertilizing duct.

x', Testis.

y, Blood-spaces.

y', Cavity between the two folds of embryo sac.

y'', Cavity of placenta.

z, Mouth.

1, 2, 3, 4, Primary blastomeres.

5, Primary cavity of follicle.

6, Proliferating area of follicle.

7, Somatic layer of follicle.

8, Visceral layer of follicle.

9, Blastoderm cells.

9', Endodermal blastomeres.

10, Placental portion of follicle.

11, Opening of fertilizing duct.

12, Blood corpuscles.

13, Pole-cell.

14, Spermatozoon.

15, Follicular cavity, or body cavity of embryo.

16, Blastopore.

17, Caudal region of embryo.

18, Caudal nerve.

19, Notochord.

20, Embryonic mesoderm.

21, Outer layer of brood sac.

22, Inner layer of brood sac.

23, Supporting ring of placenta.

24, "Blood bud."

25, Brood chamber.

26, Degenerating area of follicle.

27 and 28, Right and left tubes of the pharynx of the aggregated salpa.

29, Migrating placenta cells.

30, Caudal nerve.

31, Side walls of placenta.

33, Intercellular substance or supporting layer,

PLATE I.

1, 2, 3, 5 AND 6, SALPA PINNATA ; 4 AND 7, SALPA CHAMISSONIS.

All the figures are twice the natural size.

Figure 1, dorsal view of a fully grown specimen of the aggregated form of Salpa pinnata.

Figure 2, a young wheel-shaped cormus of Salpa pinnata, consisting of nine salpæ.

Figure 3, the same seen from the left side.

Figure 4, dorsal view of a fully grown specimen of the solitary form of Salpa chamissonis.

Figure 5, dorsal view of a fully grown specimen of the solitary form of Salpa pinnata.

Figure 6, ventral view of same.

Figure 7, ventral view of a fully grown specimen of the solitary form of Salpa chamissonis.

PLATE II.

SALPA DEMOCRATICA.

Dorsal view of a fully grown specimen of the solitary form of Salpa democratica, magnified about twenty diameters.

PLATE III.

1 AND 4, SALPA HEXAGONA ; 2 AND 3, SALPA CORDIFORMIS ; 5, 6 AND 7, SALPA CYLINDRICA.

Figure 1, dorsal view of the aggregated Salpa hexagona, magnified five diameters.

Figure 2, left side of a young free specimen of the aggregated form of Salpa cordiformis, magnified five diameters.

Figure 3, dorsal view of same.

Figure 4, an advanced embryo of Salpa hexagona, magnified twenty diameters.

Figure 5, dorsal view of the solitary form of Salpa cylindrica, magnified four diameters.

Figure 6, dorsal view of a mature specimen of the aggregated form of Salpa cylindrica, magnified four diameters.

Figure 7, ventral view of the solitary form of Salpa cylindrica, magnified four diameters.

PLATE IV.

1 AND 7, SALPA SCUTIGERA ; 2, SALPA AFRICANA ; 3, 5 AND 6, SALPA CORDIFORMIS ;
4, SALPA COSTATA.

Figure 1, part of a fully grown chain of Salpa scutigera.

Figure 2, a fully grown solitary Salpa africana.

Figure 3, a young Salpa cordiformis, magnified ten diameters.

Figure 4, a solitary Salpa costata, about half the natural size.

Figure 5, a fully grown Salpa cordiformis, enlarged three diameters.

Figure 6, part of a chain of Salpa cordiformis, enlarged five diameters.

Figure 7, an advanced embryo of Salpa scutigera, enlarged twenty diameters.

PLATES V, VI AND VII.

SALPA PINNATA.

In Plates XV, XXIII–XXXIII and XXXVI–XXXVIII, a series of sections of successive stages in the development of the aggregated Salpa pinnata, cut in planes which are parallel to the long axis of the stolon and transverse to the bodies of the salpæ, is shown.

Another stolon was cut into a series of sections at right angles to those in these plates, but in order to diminish the number of figures, I have, instead of figuring all these sections, superimposed their outlines with a camera, and combined them to form the solid pictures which are shown in Plates V, VI, VII, and Fig. 1 of Plate VIII.

In these figures the numbers at the ends of the cross lines refer to the figures of sections which correspond, or nearly correspond, to the part of the solid picture which is crossed by each line. The correspondence between the two sets of figures is not exact, for the salpæ change so rapidly during their development, that the successive stages that are exhibited by one stolon may correspond to the unrepresented intervals between the successive stages in another stolon. I have not been so fortunate as to find two well-preserved and perfect stolons which are exactly alike, but in those which are figured the departure from exact agreement is not very great, nor is it of such a character as to perplex the reader.

PLATE V.

SALPA PINNATA.

Figure 1, a proximal or dorsal view of the rudiment of a single salpa in its primary or morphological position.

Figure 2, a proximal or dorsal view of a right-hand salpa, at the stage where the morphological middle line of the oral end of the body begins to move outwards. The oral ends of the right and left pharyngeal pouches, 27 and 28, have met on the middle line, distal or ventral to the ganglion s, although each still retains its communication with the endodermal tube d' of the stolon.

Figure 3, a dorsal view of an older right-hand salpa. The oral portion of the left pharyngeal pouch, 28', has lost its connection with the aboral portion, 28, and has united behind the ganglion with the oral end of the right pouch, 27.

Figure 4, a dorsal view of a left-hand salpa, a little more advanced.

PLATE VI.

(The reference lines which are marked XXIII in Fig. 3 should be marked XXXIII.)

Figure 1, proximal or dorsal view of a right-hand salpa a little older.

Figure 2, a right-hand salpa in the foreground, and in the background a left-hand salpa partially hidden. Both are shown in proximal view.

Figure 3, the distal or far side of Figure 2. As this figure was constructed from the same series of sections, it is not reversed, but is shown as Figure 2 would appear when focused upon the far side.

Figure 4, proximal view of an older stage, showing a left-hand salpa in the foreground, and a right-hand one in the background.

PLATE VII.

Figure 1, part of the body of the right-hand salpa of Plate VI, Fig. 2, shown more enlarged in distal view.

Figure 2, a distal view of the left-hand salpa of Plate VI, Fig. 4.

Figure 3, a proximal view of a left-hand salpa at an older stage.

Figure 4, a proximal view at an older stage, showing a right-hand salpa in the foreground and a left-hand one in the background.

Figure 5, the same at a still older stage.

PLATE VIII.

1, SALPA PINNATA ; 2, SALPA CYLINDRICA ; 3, DOLIOLUM EHRENBERGII ; 4, SALPA COSTATA ; 5, SALPA CORDIFORMIS ; 6, SALPA CHAMISSONIS.

Figure 1, proximal view of two aggregated specimens of Salpa pinnata.

Figure 2, proximal view of a right-hand Salpa cylindrica in the foreground and a left-hand one in the background, at the stage shown in Plates XXXIX and XL.

Figure 3, the tailed larva of Doliolum Ehrenbergii at an advanced stage, to show the degeneration of the chorda, and the formation of a caudal vesicle or eleoblast. From Uljanin, Doliolum, Taf. 5, Fig. 1.

PLATE IX.

SALPA HEXAGONA.

(Figure 12 was drawn with a Bausch and Lomb one-eighth objective and B eyepiece. All the other figures were drawn with Zeiss D objective and A eyepiece.)

Figures 1 to 10 represent a series of sections through an egg at an early stage of segmentation, cut at right angles to the fertilizing duct. Figure 1 is the fifth section in the series; Figure 2 the seventh ; Figure 3 the eighth ; Figure 4 the ninth ; Figure 5 the tenth ; Figure 6 the eleventh; Figure 7 the fourteenth ; Figure 8 the eighteenth ; Figure 9 the twenty-fifth, and Figure 10 the thirtieth.

Figure 1 is proximal or nearest to the next younger egg in the series, and Figure 10 is distal or nearest the next older egg.

Figure 11 is a section in a plane at right angles to the plane of the sections shown above. It is from an egg at the stage which is shown in Plate X, Figs. 4 and 5.

Figure 12 is a more enlarged view of a portion of the embryo which is shown in Plate XI, Figs. 1 and 2, but the drawing was made from a section intermediate between those which are shown in the figures.

PLATE X.

EARLY STAGES IN THE EMBRYOLOGY OF SALPA HEXAGONA.

(All the figures were drawn with a Bausch and Lomb one-eighth, and were reduced one-half in the process of photographic reproduction.)

Figure 1, a section transverse to the fertilizing duct, x, of a ripe unfertilized egg.

Figure 2, a section in the same plane as Figure 1, through an egg at the time of fertilization. The head of the spermatozoon is shown just inside the egg.

Figure 3, a section through an egg after the first division, showing one of the two primary blastomeres about to divide the second time. 5 is the primary cavity of the follicle. 8 marks the point where the proliferation and migration of the follicle cells first take place. 7, the thickened and proliferating portion of the follicle. 10, the unmodified portion of the follicle. 13, pole-cells.

Figures 4 and 5, two sections from a series through an egg a little older than the one shown in Plate IX, to show the proliferation and migration of the follicle cells. 5, the primary cavity of the follicle. 7, the somatic layer of the follicle. 8, follicle cells migrating inwards to form the visceral layer of the follicle. 9, the blastomeres. 10, the area from which the follicle cells push in among the blastomeres. b, the wall of the cloaca. c, the cavity of the cloaca.

A section of another egg at nearly the same stage, but a little younger, is shown in Plate IX, Fig. 11. In this egg the migrating area of the follicle showed a distinct invagination at 6. I was not able to find this invagination in other eggs.

Figures 6, 7 and 8, three sections through an egg a little older than the one shown in Figures 4 and 5. The area 10 is now bounded by definite limits, and its cells are beginning to migrate inwards among the blastomeres.

Figure 9, a longitudinal section through an embryo at the stage shown in Plate XI, Figs. 1 and 2. The primary follicular cavity has been obliterated by the migrating follicle cells, and the somatic layer 7 of the follicle is in contact with the splanchnic layer 8. At 10 the two layers are continuous with each other. This embryo is the one which is shown at D in Fig. 10.

Figure 10, a series of four eggs and embryo at successive stages of development, from Salpa hexagona. A, an unfertilized egg ; B, an egg at the stage shown in Fig. 2; C, an embryo at the stage shown in Fig. 8 ; D, an embryo at the stage shown in Fig. 9 ; b, the thickened epithelium of the wall of the cloaca ; x, the fertilizing duct ; y, the body cavity ; 11, the secondary connection between the cloaca and the fertilizing duct.

PLATE XI.
SECTIONS OF YOUNG EMBRYOS OF SALPA HEXAGONA.

c, in Figs. 1, 2, 3, 4, 5 and 6, the cavity of the cloaca of the chain-salpa. In Figs. 3, 7 and 8, the letter c, without a reference line, also marks the cloaca of the chain-salpa, while the c with a reference line marks the branchial sac of the embryo. q, the place where the mouth is to be formed. t, the rudimentary ciliated funnel or sub-neural gland. x, fertilizing duct. y, body cavity of chain-salpa. 7, somatic layer of follicle. 8, splanchnic layer of follicle. 9, blastomeres. 9', ectodermal blastomeres. 15, secondary cavity of follicle or body cavity of embryo.

Figures 4, 5, 6, 7 and 8 are horizontal sections from the same embryo. Fig. 4 is the sixteenth section in the series ; Fig. 5 is the eighteenth ; Fig. 6 is the twenty-fourth ; Fig. 7 is the twenty-eighth ; Fig. 8 is the thirty-fifth.

PLATE XII.
HORIZONTAL SECTION THROUGH THE YOUNGEST ONE OF MY EMBRYOS OF SALPA PINNATA.

Figure 5 of Plate XVII and Figs. 1 to 9 of this plate form a series, of which Plate XVII, Fig. 5, is the sixteenth section ; Plate XII, Fig. 1, the twentieth ; Fig. 2, the twenty-fourth ; Fig. 3, the twenty-sixth ; Fig. 4, the thirty-first ; Fig. 5, the thirty-sixth ; Fig. 6, the fortieth ; Fig. 7, the forty-sixth ; Fig. 8, the fiftieth, and Fig. 9, the sixtieth. Cut B, on page 29, is a reconstruction, from this series, of a vertical transverse section, and the planes of the actual sections which are figured in this plate are

shown by the numbered parallel lines. It will be seen that these sections are not exactly horizontal, but that structures on the right of the middle plane are cut at a higher level than those on the left. As the drawings are arranged on the plate, the anterior or oral end is below.

In this plate blue marks the follicle; red, the cloaca of the chain-salpa and its derivatives; yellow, the cavity of the placenta, which is, in its origin, part of the body cavity of the chain-salpa; purple, the body cavity of the embryo, and orange, the blastomeres. (In Figs. 2, 3 and 5, the structures marked g, g' and g''' were colored orange by mistake, as they are derivatives of the follicle and not of the blastomeres.)

The positions of the various sections and the relations of the parts will be made clear by a comparison between the figures in this plate and the cut B on page 29.

PLATE XIII.

HORIZONTAL SECTIONS THROUGH AN EMBRYO OF SALPA PINNATA, A LITTLE OLDER THAN
   THE ONE SHOWN IN PLATE XII, AND OF NEARLY THE SAME AGE AS THE ONE SHOWN
   IN PLATE XLI, FIG. 1.

Figure 1 is the fifth section in the series; Fig. 2, the seventh; Fig. 3, the twelfth; Fig. 4, the fourteenth; Fig. 5, the seventeenth; Fig. 6, the twenty-second; Fig. 7, the twenty-seventh; Fig. 8, the thirty-sixth, and Fig. 9, the forty-ninth.

Cut C, on page 30, is a reconstruction, from this series, of a vertical transverse section, and the planes of the actual sections which are figured in this plate are shown by the numbered parallel lines.

Blue indicates the follicle and its derivatives; red, the cloaca of the chain-salpa and its derivatives; yellow, the body cavity of the chain-salpa and its derivatives; purple, the body cavity of the embryo, and orange, the blastomeres.

The positions of the sections and the relations of parts will be understood by comparison with cut C on page 30.

PLATE XIV.

HORIZONTAL SECTIONS THROUGH AN EMBRYO OF SALPA PINNATA, A LITTLE OLDER
   THAN THE ONE SHOWN IN PLATE XIII, AND A LITTLE YOUNGER THAN THE ONE
   SHOWN IN PLATE XXII.

Figure 1 is the twelfth section in the series; Fig. 2, the fifteenth; Fig. 3, the eighteenth; Fig. 4, the twenty-first; Fig. 5, the twenty-eighth; Fig. 6, the thirty-fourth; Fig. 7, the thirty-eighth; Fig. 8, the forty-first, and Fig. 9, the forty-ninth.

Cut D on page 31 is a reconstruction, from this series, of a vertical transverse section, and the planes of the actual sections which are figured in the plate are shown by the numbered parallel lines.

Blue indicates the follicle and its derivatives; red, the cloaca of the chain-salpa and its derivatives; yellow, the body cavity of the chain-salpa and its derivatives; purple, the body cavity of the embryo, and orange, the blastomeres.

The positions of the sections and the relations of parts will be understood by comparison with cut D on page 31.

PLATE XV.

A SERIES OF SECTIONS THROUGH A YOUNG STOLON OF SALPA PINNATA, IN A HORI-
   ZONTAL PLANE, OR A PLANE PARALLEL TO THE LONG AXIS OF THE STOLON, AND
   TRANSVERSE TO THE BODIES OF THE CHAIN-SALPÆ.

Figure 1 of Plate V is a reconstruction which was made from a series of sections at right angles to those in this plate, through a stolon at about the same stage of development.

The red marks the endodermal tube of the stolon and the endoderm of the chain-salpa; the yellow, the blood tubes of the stolon and the body cavities of the chain-salpa; the blue, the follicle and its derivatives; the purple, the ectoderm and ganglion; the orange, the eggs, and the green, the perithoracic tubes.

At this stage the bodies of the chain-salpæ lie in their primary positions, and the middle plane of the stolon is also the middle line of each salpa, as is shown by Plate V, Fig. 1. At the top, Fig. 10, and bottom, Fig. 1, of the stolon, the body cavities of the salpæ are completely shut in by the ectoderm, but in all the other sections they communicate with the blood tubes of the stolon.

Figure 1, a section close to the ventral surface of the stolon showing the testicular folds m, m of the follicle.

Figure 2, a section a little higher up, showing the series of eggs, n, partially separated from each other by infoldings of the follicle.

Figures 3 and 4, sections through the ventral blood tube.

Figure 5 cuts the left perithoracic tube (green) and the left half of the pharynx (red).

Figure 6: the left half of the pharynx, 28, is cut in the plane of its communication with the endodermal tube, d', of the stolon, while the right half of the section lies below the endodermal tube and cuts the ventral blood tube, i.

Figure 7: a section in the plane of the endodermal tube, d', of the stolon, showing its communication with both the left half of the pharynx, 28, and the right half, 27. The section passes through the right perithoracic tube, g, and the heart, e, but it passes above the left perithoracic tube.

Figure 8: the right side is nearly the same as in Fig. 7, but on the left the section cuts the dorsal blood tube, j', and the dorsal elongation of the left half of the pharynx, 28.

Figure 9: the right half of the section cuts the tips of the dorsal elongations of the right tubes of the pharynx.

Figure 10: a section through the ganglion, s, close to the top of the stolon.

Figures 11 and 12, parts of sections 5 and 6, more enlarged.

Figure 1 is the first section in the series; Fig. 2, the sixth; Fig. 3, the eighth; Fig. 4, the fourteenth; Fig. 5 is not in the same series; Fig. 6 is the twentieth; Fig. 7 is the twenty-fifth; Fig. 8, the twenty-ninth; Fig. 9, the forty-fourth, and Fig. 10, the fifty-fifth. All the figures except Fig. 5 and Figs. 11 and 12 are drawn with the top distal or towards the tip of the stolon, and with its right side on the right of the figure.

PLATE XVI.

Figures 1, 2 and 3 are in the same series with Figs. 1, 2, 3, and 4 of Plate XVII. They are horizontal sections of an embryo of Salpa pinnata, a little older than the one shown in Plate XXII.

Figure 1 is the thirty-sixth section in the series; Fig. 2, the forty-fourth; Fig. 3, the fifty-third; Fig. 4 of Plate XVII, the sixtieth; Fig. 2 of Plate XVII, the sixty-sixth; Fig. 3, the seventy-fourth and Fig. 4, the sixty-first.

Figure 4 of Plate XVI is a transverse section through the stolon and endostyle of an embryo, at the stage which is shown in Plate XLI, Figs. 5 and 6.

Figure 5 is a longitudinal section of a stolon from an embryo at the stage shown in Plate XLI, Figs. 3 and 4.

Figures 6 and 7 are two stages in the history of the ectoderm; Fig. 7, from the embryo, shown in Fig. 2 of this plate, and Fig. 6, from a younger embryo.

PLATE XVII.

Figures 1, 2, 3, and 4 are described in the explanation of Plate XVI.

Figure 5 is described in the explanation of Plate XII.

Figures 6 and 7: two stages in the formation of the cloacal aperture of the embryo of Salpa pinnata; Fig. 6, from the embryo, shown in Plate XXXV, and Fig. 7, from an older embryo.

PLATE XVIII.

A SERIES OF TRANSVERSE SECTIONS FROM AN EMBRYO OF SALPA PINNATA, A LITTLE YOUNGER THAN THE ONE SHOWN IN PLATE XLI, FIG. 2.

Figures 1 to 7: vertical transverse sections.

Figure 8: vertical longitudinal section.

29, migrating follicle cells from the roof of the placenta.

PLATE XIX.

A SERIES OF HORIZONTAL SECTIONS FROM AN EMBRYO OF SALPA PINNATA, A LITTLE YOUNGER THAN THE ONE SHOWN IN LONGITUDINAL SECTION IN PLATE XXXV.

Figure 1 is a section in the plane of the gill, o, and œsophagus, g.

Figure 2 is a section which passes through the ganglion, s.

Figure 3 is a section through the subneural gland, t, and the opening of the endodermal tube, d', of the stolon into the pharynx, c, between the halves of the endostyle, d, d.

Figure 4 passes through the eleoblast, k, and the endodermal tube of the stolon, d'.

Figure 5 passes through the germinal mass, and cuts the endodermal tube of the stolon below its opening into the pharynx.

Figure 6 shows the heart, c, of Fig. 1, more enlarged.

Figure 7 is a mass of follicle cells from a section between 2 and 3.

Figure 8 is the heart of Figure 2.

Figure 9 is the eleoblast, k; the pericardium, f; the heart, c, and the endodermal tube, d', of Fig. 3.

Figure 10 is the same region of Figure 5.

PLATE XX.

LONGITUDINAL AND TRANSVERSE SECTIONS OF YOUNG STOLONS OF SALPA PINNATA.

All the figures were drawn with a Bausch and Lomb ⅛ objective and A eyepiece, and they have been reduced one-half in photolithography.

Figs. 1, 2, 3 and 4 are reversed, as the pericardium, f, is actually on the right side of the stolon instead of the left.

Figures 1, 2, 3 and 4, transverse sections in a series from an embryo at about the stage shown in Plate XIX and in Plate XLI, Fig. 4.

Figure 1 is the fifth section in the series; Fig. 2 is the ninth; Fig. 3 is the fourteenth, and Fig. 4 the twenty-third. Figs. 5, 6 and 7 are longitudinal sections of very young stolons, and Fig. 6 is the youngest; Fig. 5 next, and Fig. 7 oldest. Fig. 7 is from an embryo at the stage shown in Plate XXXV. Fig. 5 is shown very highly magnified in Plate XLI, Fig. 7.

REFERENCE LETTERS FOR PLATES XX AND XXI.

a, ectoderm of the embryo. a', ectoderm of the stolon. a'', layer of ectoderm reflected to form a sheath around the base of the stolon. b, endoderm of pharynx of

embryo. *c*, cavity of pharynx of embryo. *d*, endostyle of embryo. *d'*, endodermal tube of stolon. *e*, heart of embryo. *f*, pericardium. *g*, right perithoracic tube of stolon. *h*, left perithoracic tube of stolon. *l*, ganglion. *m*, follicle. *n*, ovary.

PLATE XXI.

A Series of Transverse Sections of a Young Stolon of Salpa Pinnata, at about the stage shown in Plate XLI, Figs. 5 and 6.

All the figures were drawn with a Bausch and Lomb ⅛ and A eyepiece, and are reduced one-half in photolithographing.

Figure 1 is nearest the root or proximal end of the stolon, and is the first section in the series; Fig. 2 is the seventh; Fig. 3 is the eleventh; Fig. 4 is the fourteenth; Fig. 5 is the seventeenth; Fig. 6 is the twentieth, and Fig. 7 is the twenty-ninth.

a, ectoderm of embryo. *a'*, ectoderm of stolon. *a''*, reflected layer of ectoderm. *b*, endoderm of pharynx of embryo. *c*, cavity of pharynx. *d*, endostyle of embryo. *d'*, endodermal tube of stolon. *e*, heart of embryo. *f*, pericardium of embryo. *g*, right perithoracic tube of stolon. *h*, left perithoracic tube of stolon. *i*, blood space between the endodermal tube and the genital string of the stolon. In the plane of sections 1 and 2 this communicates with the eleoblast, *k*, of the embryo. *j*, blood space between the endodermal tube and the nerve tube of the stolon. In the planes of Figs. 3 and 4 this communicates with the heart, *e*, of the embryo. *k*, eleoblast. *m*, follicle. *n*, ovary.

PLATE XXII.

Transverse Sections through an Embryo of Salpa·Pinnata, at the stage shown in Plate XIV.

While the sections in this plate are symmetrical, they are not quite perpendicular to the plane of the horizontal sections in Plate XIV, but parallel to a plane which is inclined forwards, so that they make an acute angle in front and an obtuse angle behind with the plane of Plate XIV. Cut D, on page 31, is a perpendicular transverse section at this stage.

a'', ectoderm rudiment. *b'*, epithelial capsule. *c*, cavity of pharynx. *g'*, perithoracic tubes. *g''*, remains of spiracles. *g'''*, cloaca. *s*, ganglion. *y*, blood space of chain-salpa. *y'*, space between the folds of the embryo sac. *y''*, cavity of placenta. 7, somatic layer of follicle. 8, visceral layer of follicle. 10, placental portion of follicle. 21, outer fold of embryo sac. 22, inner fold of embryo sac. 23, supporting ring. 24, blood bud. 25, brood chamber. 26, migrating follicle cells.

PLATES XXIII AND XXIV.

In these plates, and in all the figures of sections parallel to the long axis of the stolon, the proximal surface is at the bottom, and the right side on the right of the figure. Figures 1, 2, 3 and 4 of Plate XXIII, and 1, 2, 3, 4 and 5, XXIV, are from a series of horizontal sections of a stolon of Salpa pinnata, at the stage which is shown in cut N on page 81.

The two pharyngeal pouches have reached the oral end of the body, and the salpæ are more completely marked out in the walls of the stolon than they were at an earlier stage.

Figure 1 of Plate XXIII is the fourth section in the series; 2 is the sixth; 3, the eighth, and 4, the·thirteenth. In Plate XXIV, Fig. 1 is the thirty-fourth; 2, the thirty-seventh; 3, the forty-second; 4, the fifty-second, and 5, the sixty-second.

Figures 5 to 10 of Plate XXIII are horizontal longitudinal sections of a stolon of Salpa pinnata, at a stage a little later than Plate XXXIV, Figs. 8, 9, 10 and 11. Fig. 5 is the second section in the series; 6, the thirty-third; 7, the thirty-seventh; 8, the right half; 9, the left half of the fortieth, and 10, the forty-fourth.

PLATES XXV TO XXXI.

SALPA PINNATA.

A series of sections, longitudinal to the stolon and transverse to the bodies of the salpæ, showing the structure and the anatomical relations of eight salpæ at successive stages of development. The capital letters mark the same salpæ in successive sections, and the letters with an accent mark their left sides.

In all the figures the right side of the stolon is on the right side of the plate, and as the top of the figure is distal the upper salpa in each figure is oldest. As the body of an old salpa is longer than that of a young one, it is of course cut by more sections, and is shown in more figures. The structure which is marked g in Plate XXV, Fig. 4, H; Fig. 7, E and F; Plate XXVI, Fig. 1, F, and Plate XXVII, Fig. 2, C, is the *digestive tract*, and not the *right perithoracic vesicle*, which is marked g in the other figures.

PLATE XXV.

Figure 1 is the second section in the series; 2 is the third; 3, the fourth; 4, the sixth; 5, the seventh; 6, the eighth, and 7, the tenth.

PLATE XXVI.

Figure 1 is the eleventh section in the series; 2, the twelfth, and 3, the fourteenth.

PLATE XXVII.

Figure 1 is the fifteenth section of the series; Fig. 2, the sixteenth, and Fig. 3, the seventeenth.

PLATE XXVIII.

Figure 1 is the nineteenth section in the series, and Fig. 2, the twentieth.

PLATE XXIX. ·

Figure 1 is the twenty-first section in the series; Fig. 2 is the twenty-third; Fig. 3 is the right half of salpa, $B–B'$, in section twenty-five; Fig. 4 is the same in section twenty-seven; Fig. 5 is the same in section twenty-eight; Fig. 6 is the same in section twenty-ninth; Fig. 7 is the left half of the $B–B'$ in section twenty-seven, and Fig. 8 is the same in section twenty-nine.

PLATE XXX.

Figure 1 is the thirtieth section in the series; 2 is the thirty-seventh, and Fig. 3, the forty-second.

PLATE XXXI.

Figure 1 is the forty-ninth section in the series; 2 is the sixtieth, and 3, the sixty-fifth.

Figures 4, 5, 6, 7 and 8 are sections through the genital rod of a stolon a little older than the one which is shown in Plate XLI, Fig. 6.

Figure 9 is from a young stolon which had traces of constriction of the ectoderm at its tip.

PLATES XXXII AND XXXIII.

A series of sections transverse to the long axis of the bodies of six aggregated salpæ of Salpa pinnata, at the stages which are shown in Plate VI, Figs. 2, 3 and 4, and Plate VII, Figs. 1 and 2. The structure which is colored blue in Plate XXXII, Figs. 6 and 7, K and Fig. 4, M, is the intestine, and the structure marked g in Figs. 4, M; 5, M; 7, K and 8, K, is the œsophagus.

PLATE XXXIV.

Salpa Pinnata.

Figure 1, a transverse section near the root of a stolon from a mature salpa which has produced great numbers of buds.

Figure 2, the genital rod, more enlarged.

Figure 3, the region of the right perithoracic tube, more enlarged.

Figure 4, a horizontal longitudinal section through the genital rod of the same stolon, a little farther from the root.

Figure 5 and Fig. 6, two sections through the endodermal tube and the right perithoracic tube of the same stolon.

Figure 7, a section above the level of the perithoracic tube.

Figures 8, 9, 10 and 11, sections through the perithoracic tubes of more advanced stages, to show the way in which the perithoracic tubes and the endodermal tube are cut by the folds of ectoderm.

PLATE XXXV.

Median longitudinal section through an embryo of Salpa pinnata, about the stage shown in Plate XLI, Fig. 3.

a, ectoderm. a', ectoderm of stolon. b, endoderm. c, pharynx. d', endodermal tube of stolon. e, heart. f, pericardium. g''', cloaca. g^v, cloacal aperture. k, eleoblast. n, germinal mass of stolon. o, gill. p, intestine. p', stomach. p'', anus. s, ganglion. t, ciliated funnel. v, cellulose mantle. w, oral muscles. y', blood spaces of amnion. y'', blood spaces of placenta. z, mouth.

10, roof of placenta. 15, body cavity. 21, outer fold of amnion. 22, inner fold of amnion. 23, supporting ring of placenta.

PLATE XXXVI.

Salpa Pinnata.

Transverse sections through two aggregated salpæ, a right-hand and a left-hand one, at the stage which is shown in Plate VII, Fig. 3.

Figure 1 is the third section in the series; 2 is the fifth; 3 is the seventh; 4, the tenth; 5, the eleventh; 6, the thirteenth; 7, the sixteenth; 8, the twentieth; 9, the twenty-third; 10, the twenty-seventh; 11, the thirtieth; 12, the thirty-second; 13, the thirty-fourth; 14, the thirty-eighth; 15, the forty-third, and 16, the forty-eighth.

PLATE XXXVII.

1-24, Salpa Pinnata. 25 and 26, Salpa Africana.

Figures 1–14 are transverse sections through two aggregated salpæ, at the stage which is shown in Plate VII, Fig. 4.

Figures 15–24 are at the stage which is shown in Plate VII, Fig. 5.

In the first series Fig. 1 is the first section; Fig. 2, the second; 3, the third; 4, the fifth; 5, the sixth; 6, the seventh; 7, the eighth; 8, the eleventh; 9, the twenty-

first ; 10, the twenty-eighth ; 11, the thirty-third ; 12, the thirty-fifth ; 13, the thirty-eighth, and 14, the fiftieth.

In the second series Fig. 15 is the sixth section ; 16, the ninth ; 17, the twelfth ; 18, the twentieth ; 19, the thirty-first ; 20, the thirty-sixth ; 21, the forty-third ; 22, the forty-sixth ; 23, the forty-ninth, and 24, the fifty-second.

Figure 25, the sections of Salpa africana which are shown on a smaller scale at 1, 2 and 3 of cut Y on page 107.

Figure 26, the section of Salpa africana which is shown on a smaller scale at 4 in cut Y on page 107.

PLATE XXXVIII.

SALPA PINNATA.

Two aggregated salpæ, a right-hand and a left-hand one, at the stage which is shown in Plate VIII, Fig. 1.

PLATES XXXIX AND XL.

SALPA CYLINDRICA.

These sections are from a stolon of Salpa cylindrica at the stage shown in Plate VIII, Fig. 2.

The figures are placed with their proximal surface below, like the plates of Salpa pinnata, but the first one in the series, Fig. 1, instead of being at the lower left-hand corner, is at the upper right-hand corner. The series included nearly two hundred sections, and those which were drawn were selected for the purpose of giving a consecutive series in as small a number of figures as possible, and while the right-hand ones are not cut in exactly the same plane as the left-hand ones, the difference is not very great, and its nature is easily understood from Plate VIII, Fig. 2. With this slight difference the following series gives what is in effect a more complete series from a single salpa :

Fig. 1, right. Fig. 2, right. Fig. 3, right. Fig. 4, right. Fig. 5, right. Fig. 6, right. Fig. 7, right. Fig. 1, left. Fig. 2, left. Fig. 3, left. Fig. 4, left. Fig. 5, left. Fig. 6, left. Fig. 7, left. Fig. 11 and Fig. 12, left. Fig. 13, left. Fig. 14, left. Fig. 19, right. Fig. 20, right. Fig. 21, right. Fig. 17, left. Fig. 18, left. Fig. 19, left. Fig. 20, left. Fig. 21, left. Figs. 21 and 22.

PLATE XLI.

Figure 1, embryo of Salpa pinnata at about the same stage as Plate XIV, Fig. 2. 25, cavity of amnion. 23, supporting ring of placenta. 24, blood bud. y', body cavity of chain-salpa. y'', blood space of placenta.

Figure 2, embryo of Salpa pinnata a little older than Plate XVIII. g''', cloaca. g^v, place where cloacal aperture is formed at a later stage. f, pericardium. c, pharynx. s, ganglion. v, cellulose. z, mouth. y'', blood space of placenta. 21, outer, 22, inner fold of amnion. 23, supporting ring. 24, blood bud. x is place of stolon. k, of eleoblast.

Figure 3, embryo of Salpa pinnata a little older than in Plate XXXV, with the stolon in the stage shown in Plate XX. g''', cloaca. g^v, cloacal aperture. k, eleoblast. o, gill. p, intestine. p^2, placenta at stolon. z, mouth.

Figure 4, stolon of Fig. 3 more highly magnified. d', digestive tube of stolon. a, ectoderm. a', ectoderm of stolon. n, germinal mass.

Figure 5, embryo of Salpa pinnata with stolon in stage shown in Plate XXI. Reference letters as in Fig. 3.

Figure 6, stolon of Fig. 5 more highly magnified.

Figure 7, the germinal mass at the stage shown in Plate XX, Fig. 5, drawn with a Tolles $\frac{1}{12}$, oil immersion. *a*, ectoderm. *b*, endoderm. *c*, pharynx. *f*, pericardium. *n*, germinal mass. *y*, body cavity. *A*, blood corpuscles and mesoderm cells. *B*, follicle cells.

Fig. 8, section through genital string at the root of a mature stolon.

Fig. 9, section through mature stolon a short distance from root. These two sections are from the same series as the figures in Plates V, VI, VII and VIII.

Figure 10, a wheel-shaped cormus of Salpa chamissonis.

PLATE XLII.

Salpa Hexagona and Salpa Pinnata.

(Tolles $\frac{1}{12}$, homogeneous immersion.)

Figure 1, part of the embryo of Salpa hexagona shown in Plate XI, Fig. 1, from another section in the same series. 7, somatic layer of follicle. 8, visceral layer of follicle. 9, blastomeres.

Figure 2, part of the embryo of Salpa hexagona shown in Plate XI, Fig. 3, from another section in the same series.

Figure 3, part of the section of Salpa pinnata shown in Plate XII, Fig. 7.

Fig. 4, part of the embryo of Salpa pinnata shown in Plate XIII, Fig. 7, but from the next section.

Figure 5, part of a section of Salpa pinnata, between 7 and 8 of Plate XIV.

Figures 6, 7 and 8 are from an embryo of Salpa pinnata a little older than Plate XIV, cut in the plane of cut D, page 31. *g*, median atrium, or cloaca. *g''*, gill-slits. *c*, pharynx. *y*, body cavity. *o*, gill. 7, somatic layer of follicle. 8, visceral layer of follicle. 9, blastomeres.

Figure 9, part of the section of Salpa pinnata shown in Plate XVI, Fig. 3. *a*, ectoderm. *b*, epithelial lining of gill-slits. *b'*, cells of epithelial capsule. *g*^{IV}, gill-slit. *A*, mesoderm cells. 8, follicle cells in body cavity. *X*, follicle cells in cavity of atrium.

Figure 10, part of Plate XVII, Fig. 2. *c*, cavity of pharynx. *q*, rudiment of gut.

Figure 11, ectodermal rudiment of an embryo of Salpa pinnata at the stage of cut D, page 31. *a*, ectoderm. *b'*, cells of epithelial capsule. 8, follicle cells. 15, body cavity.

PLATE XLIII.

Salpa Democratica and Salpa Runcinata.

Figure 1, part of a fully grown chain of Salpa democratica.

Figure 2, the aggregated form of Salpa runcinata.

PLATE XLIV.

Figure 1, a section through the eleoblast of an embryo of Salpa hexagona at the stage shown in Plate III, Fig. 4.

Figures 2, 3 and 4, three horizontal sections through the placenta of an embryo of Salpa pinnata at the stage shown in Plate XXXV. Fig. 2 is at the level of the letters *io* in Plate XXXV; Fig. 3, at the level of the letters *bl.* 2; and Fig. 4, at the letter *y''*. Fig. 5 is a portion of Fig. 3 more highly magnified.

PLATE XLV.

SALPA PINNATA AND SALPA HEXAGONA.

Figure 1, a longitudinal section through the placenta of the embryo of Salpa hexagona at a very early stage.

Figures 2, 3 and 4, transverse sections through three embryos of Salpa hexagona, at three successive stages.

Figures 6 and 7, sections of young aggregated salpæ of Salpa hexagona from a stolon ; N, the eggs.

Figure 5, a transverse section through the endostyle and stolon of Salpa pinnata, at the stage shown in Plate XLI, Fig. 5.

PLATE XLVI.

SALPA PINNATA AND SALPA AFRICANA.

Figures 1 and 2, two longitudinal sections through an advanced stolon of Salpa pinnata, to show the changes in the positions of the salpæ.

Figure 3, a more enlarged figure of the sections 6 and 7 of cut Y on page 107.

EXPLANATION OF PLATES XLVII TO LVIII.

COLORS.

The *dark blue ink* in which the plates are printed represents those portions that stain deeply with Kleinenberg's hæmatoxylin, *i. e.*, cell walls, basement membranes, chromatin fibers of nuclei, protoplasmic granules, etc.

Light blue = central nervous system, also certain mesoderm elements, blood corpuscles and spindle cells, in the neighborhood of the ganglion.

Grayish violet = ectodermal epithelium.

Light green = epithelium of the wall of the pharynx or cloaca and of the subneural gland. Owing to a mistake in the final printing, this color is hardly distinguished from the too deep yellow.

Yellow = body cavity, usually more or less filled with coagulum and scattered connective tissue elements. The larger blood spaces are frequently left uncolored.

Reddish brown = pigment.

REFERENCE LETTERS.

a = lateral lobe of the dorsal eye of Salpa cylindrica.

an = antero-dorsal angle of the ganglion in Salpa scutigera-confederata, or its homologue in other species.

b = small celled, ventro-lateral outgrowth from the ganglion toward h.

b' = large celled, ventro-lateral outgrowth from the ganglion toward h.

bc = blood corpuscle.

bl = blood lacuna.

cf = ciliated funnel.

cr = non-cellular core of the optic ridge.

d = lateral duct of the subneural gland.

d' = remnant of the right lateral duct of the subneural gland in Salpa cylindrica, chain form.

dl = dorsal lamina (gill or its anterior curtain-like projection).

do = opening of the lateral duct of the subneural gland into the branchial chamber.

e = eye or rudiment of the eye.

e' = basal portion of the unpaired eye of the chain form.

$e'\,1$, $e'\,2$ = two portions of e', found in Salpa democratica-mucronata, chain form.

e'' = apical portion of the unpaired dorsal eye of the chain form.

e''' = secondary portion of the unpaired dorsal eye of the chain form.

eq = antero-ventral enlargement of the retina of the solitary Salpa runcinata-fusiformis.

ex = one of the dorsal pair of small eyes in the chain Cyclosalpa pinnata, or its homologue in other species.

ey = one of the posterior pair of small eyes in the chain Cyclosalpa pinnata, or its homologue in other species.

f = canal of the central nervous system.

f' = communication between f and l.

$f\,1$ = canal of the duct between the ganglion and the funnel.

g = ganglion.

Figures 11–12, transverse sections of the ventral portion of the same ganglion shown in Fig. 10. Fig. 12 represents the more anterior section. The disk-shaped gland is shown at *h*, and one of the ventro-lateral outgrowths from the ganglion at *b*. × 180 diameters.

PLATE XLIX.

CYCLOSALPA PINNATA, *Chain Form, Adult.*

Figures 1 and 2, longitudinal, vertical sections of the ganglion and unpaired eye. Fig. 2 represents the section nearer the mid-line. The three regions of the unpaired eye are shown, *e'*, *e''*, *e'''*. × 157 diameters.

Figure 3, part of a horizontal section through the region marked *e'* in Fig. 2. It shows the thick-walled ends of the rod cells in cross-section. The nucleus-like bodies (secondary nuclei) should be more homogeneous.

Figure 4, a transverse section of the ganglion through the region marked *on* in Fig. 1. × 157 diameters.

Figure 5, a more anterior section from the same series, cutting the eye through the region marked *e'* in Fig. 2. × 157 diameters.

Figure 6, a more enlarged drawing of the right limb of the eye shown in Fig. 4. × 278 diameters.

Figure 7, an optical section of a piece of the second, smaller portion of the unpaired eye, separated by teasing and having some of the fibers of the optic nerve still attached. × 157 diameters.

Figure 8, a longitudinal, vertical section of the antero-dorsal half of the ganglion, showing one of each pair of smaller eyes, *ex*, *ey*. The position of the large unpaired eye, *e'*, is indicated. × 481 diameters.

Figure 9, the nerve plexus on the dorsal surface of the ciliated funnel. × 265 diameters.

PLATE L.

SUBNEURAL GLAND OF CYCLOSALPA PINNATA, *Chain Form.*

Figure 1, a longitudinal, vertical section of the ganglion at a stage of development corresponding to that represented in Plate XLVII, Fig. 2, showing the commencement of the formation of the lateral tubes of the subneural gland, *h*. The section is on one side of the middle line. × 240 diameters.

Figure 2, an oblique section of the ganglion at the same stage of development as that shown in Fig. 1, showing part of the remnant of the duct from the ganglion to the ciliated funnel, *v*. × 240 diameters.

Figure 3, a longitudinal section of the ganglion at a stage of development corresponding to Figs. 5 and 6, Plate XLVII, showing a later stage in the formation of the lateral tubes of the subneural gland. × 240 diameters.

Figures 4–6, longitudinal sections, not quite vertical, of the adult ganglion and the adjacent parts, showing the subneural gland, *d* and *h*, and the ventro-lateral outgrowths from the ganglion, *b* and *b'*. Fig. 4 is nearer the mid-line. × 157 diameters.

Figure 7, a longitudinal section of the disk-like enlargement of the neural end of one of the lateral tubes. × 408 diameters.

Figures 8–11, transverse sections of the ventral half of the ganglion and the adjacent parts, showing the relation of the subneural gland to the ventro-lateral outgrowths from the ganglion. Fig. 11 is the more anterior. × 157 diameters.

Figure 12, two cells from the large-celled ventro-lateral outgrowth from the ganglion, shown in Fig. 11 at *b'*. The actual diameter of the cell is .015 mm.; the actual diameter of the nucleus is .0065 mm.

PLATE LI.

PAIRED EYES OF CYCLOSALPA PINNATA, *Chain Form.*

Figure 1, a transverse section of the posterior part of the ganglion, showing the posterior pair of small eyes, *ey.* × 240 diameters.

Figures 2 and 3, transverse sections of the dorsal part of the ganglion, showing the dorsal pair of small eyes, *ex.* × 240 diameters.

Cf. also Figure 8, Plate XLIX.

CYCLOSALPA PINNATA, *Solitary Form.*

Figure 4, a longitudinal, vertical section of the ganglion of a young embryo, showing how the dorsal part of the adult ganglion is formed from the dorsal wall of the originally thin-walled neural canal. × 265 diameters.

Figures 5 and 6, transverse sections through the rudiment of the optic ridge, *e,* and the ganglion with adjacent parts. Figure 5 shows the two anterior limbs of the eye; Fig. 6 its posterior curved portion. × 157 diameters.

Fig. 7, a transverse section through the ganglion, eye, and adjacent parts in an advanced embryo, showing also the disk-like enlargements of the lateral tubes of the subneural gland. The section cuts the two anterior limbs of the eye. × 157 diameters.

Figure 8, a transverse section through the ganglion and the anterior limbs of the eye. Adult. × 157 diameters.

Figure 9, a more enlarged drawing of one of the limbs of the eye shown in Fig. 8. × 495 diameters.

PLATE LII.

SALPA CYLINDRICA, *Solitary.*

Figure 1, a transverse section of the ganglion, eye and adjacent parts. The section cuts the posterior curved part of the eye. × 240 diameters.

Figure 2, a similar section cutting the two anterior limbs of the eye. × 157 diameters.

Figure 3, a more enlarged drawing of one of the two limbs of the eye shown in Fig. 2. × 495 diameters.

Chain Form.

Figure 4, a longitudinal, vertical section of the ganglion, eyes, ciliated funnel and adjacent parts, showing the two eyes, *ex* (the smaller) and *e' + e''* (the larger). × 98 diameters.

Figures 5–7, transverse sections of three regions of the larger eye. The small lateral lobes of the eye are seen at *a.* Fig. 5 represents the more posterior section. × 240 diameters.

Figure 8, a transverse section of the ganglion, through the small eye, *ex,* showing also the slight ventro-lateral outgrowths from the ganglion, *b.* × 240 diameters.

Figure 9, a transverse section of the dorsal part of the ganglion, through the center of the smaller eye, showing its innervation, *on.*

Figures 10 and 11, transverse sections of the parts adjacent to the ganglion, showing the well-developed left lateral tube, *d,* and the rudimentary right lateral tube, *d',* of the subneural gland. × 180 diameters.

Figure 12, a longitudinal, vertical section of the ganglion and the adjacent parts, showing a portion of the left lateral tube of the subneural gland. × 180 diameters.

SALPA HEXAGONA, *Chain Form.*

Figure 13, a longitudinal, vertical section of the ganglion and eye.   × 157 diameters.

Figure 14, a transverse section through the basal portion of the eye.

Figure 15, a transverse section through the ganglion, showing the lateral outgrowths of rod-like cells.   × 180 diameters.

PLATE LIII.

CYCLOSALPA CHAMISSONIS, *Chain Form.*

Figures 1 and 2, transverse sections through the large dorsal eye ; Fig. 1, a section through the posterior region ; Fig. 2, a section through the junction of the secondary smaller portion of the eye and the anterior part of the main body of the eye.   × 180 diameters.

SALPA RUNCINATA-FUSIFORMIS, *Solitary Form.*

Figure 3, a longitudinal, vertical section through the ganglion and one limb of the eye.   × 197 diameters.

Figures 4–6, transverse sections of the ganglion and eye ; Fig. 6 representing the more anterior section.   × 180 diameters.

Chain Form.

Figure 7, a longitudinal, vertical section of the unpaired eye and the anterior part of the ganglion.   The smaller eye is also shown (*ex*).   × 265 diameters.

Figure 8, a transverse section of the basal portion of the larger eye.   × 495 diameters.

Figure 9, a transverse section of the ganglion through the smaller eye, *ex*.   It shows also the antero-ventral outgrowth from the ganglion, *v*.   × 157 diameters.·

PLATE LIV.

SALPA AFRICANA-MAXIMA, *Chain Form.*

Figure 1, a transverse section of the base of the larger eye.   × 265 diameters.

Figure 2, two depigmented pigment cells of the latter, more highly magnified.

Figures 3–8, transverse sections of the ventral part of the ganglion and of the antero-ventral outgrowth from the same.   Figure 3 represents the more posterior section.   × 180 diameters.

Figure 9, a transverse section of the ganglion and the subneural gland.   Of the lateral ducts only the right one is shown.   × 180 diameters.

SALPA COSTATA-TILLESII, *Chain Form.*

Figure 10, a longitudinal vertical section of the ganglion and the large eye. The smaller masses of rod cells are also shown, *ex, ey*.   × 98 diameters.

SALPA CORDIFORMIS-ZONARIA, *Chain Form.*

Figures 11 and 12, longitudinal, vertical sections of the ganglion, eye and ciliated funnel.   The antero-ventral outgrowth from the ganglion, *v*, is well shown.

Figure 13, a longitudinal, vertical section of the eye.   × 476 diameters.

Solitary Form.

Figures 14 and 15, transverse sections of the ganglion and eye.   Fig. 14 cuts the two anterior limbs ; Fig. 15 the posterior curved portion.   The large ventro-lateral outgrowths from the ganglion, *vl*, are shown.   × 300 diameters.

PLATE LV.

SALPA SCUTIGERA-CONFEDERATA, *Chain Form.*

Figure 1, a longitudinal, vertical section of the ganglion, eye and ciliated funnel. 81.1 diameters.

Figure 2, a longitudinal, vertical section of the ganglion and eye. × 180 diameters.

Figures 3–5, transverse sections of the ganglion and adjacent parts, showing the larger eye, the smaller masses of rod cells (*y*), the ventro-lateral outgrowths from the ganglion (*b'*), and portions of the subneural gland (*h* and *d*). Fig. 5 represents the more anterior section. Fig. 3 × 98 diameters ; Figs. 4 and 5 × 180 diameters.

SALPA BICAUDATA, *Chain Form.*

Figure 6, a transverse section of the two lobes of the eye. × 180 diameters.

Figure 7, a longitudinal, vertical section of the ganglion and eye. × 180 diameters.

Figures 8 and 9, transverse sections of the ganglion and adjacent parts, showing the subneural gland (*h*) and the ventro-lateral outgrowths from the ganglion (*b'*). × 180 diameters.

SALPA DEMOCRATICA-MUCRONATA, *Solitary Form.*

Figure 10, a longitudinal, vertical section of the ganglion and eye, showing also one of the disk-like portions of the subneural gland, *h*. × 240 diameters.

Figures 11 and 12, transverse sections of the ganglion and eye. Fig. 11 cuts the posterior part of the eye ; Fig. 12 the two anterior limbs. × 240 diameters.

Chain Form.

Figure 13, a longitudinal, vertical section of the ganglion, eye and ciliated funnel. × 98 diameters.

Figures 14 and 15, longitudinal, vertical sections of the ganglion and eye. × 300 diameters.

PLATE LVI.

Figure 1, a postero-dorsal view of the ganglion and eye of Salpa bicaudata, chain form. × 58½ diameters.

Figure 2, a dorsal view of the ganglion and eye of Salpa costata-Tillesii, chain form. × 24½ diameters.

Figure 3, a dorsal view of the eye, ciliated funnel, ganglion and nerves of Salpa cordiformis-zonaria, chain form. × 52 diameters.

Figure 4, a dorsal view of the ganglion, eye and ciliated funnel of Salpa cylindrica, chain form. × 173 diameters.

Figure 5, a dorsal view of the eye, ganglion, some of the nerves and a portion of the ciliated funnel of Salpa cordiformis-zonaria, solitary form. × 70 diameters.

Figure 6, a dorsal view of the ganglion, eye, and a portion of the ciliated funnel of Salpa runcinata-fusiformis, chain form. The ganglion and eye are twisted so that the view is somewhat from the left side and not strictly dorsal. × 173 diameters.

Figure 7, a dorsal view of the eye, ganglion, nerves and ciliated funnel of Salpa scutigera-confederata, solitary form. × 60 diameters.

PLATE LVII.

Figure 1, a longitudinal, vertical section of the ganglion and adjacent parts of a young embryo (solitary form) of Salpa hexagona, showing the peculiar fold of ectoderm back of the ganglion. × 215 diameters. *In this figure the letters al should read dl.*

Figure **2**, a transverse section of the same structures.   × 215 diameters.

Figure 3, a diagrammatic surface view of the same structures in a very early stage of development.

Figure 4, a diagrammatic surface view of these structures in a later stage of development.

Figure 5, a dorsal view of the ganglion and eye of Salpa hexagona, chain form, showing the lateral outgrowths from the ganglion.   × 118 diameters.

Figure 6, a dorsal view of the ganglion and eyes of Salpa democratica-mucronata, chain form.   × 362 diameters.

Figure 7, a dorsal view of the eye, ganglion, nerves and ciliated funnel of Salpa democratica-mucronata, solitary form.   × 70 diameters.

Figure 8, a lateral view of the ganglion and dorsal eye of Cyclosalpa Chamissonis, chain form.   × 118 diameters.

Figure 9, a dorsal view of the eye, ganglion and nerves of Cyclosalpa pinnata, chain form.   × 118 diameters.

Figure 10, a lateral view of the same.   × 118 diameters.

SALPA.

PLATE I.

Fig 1.

Fig 2.

Fig 3

Fig. 4.

Fig. 5.

Fig. 6.

Fig. 7.

W.K.BROOKS, autograph.
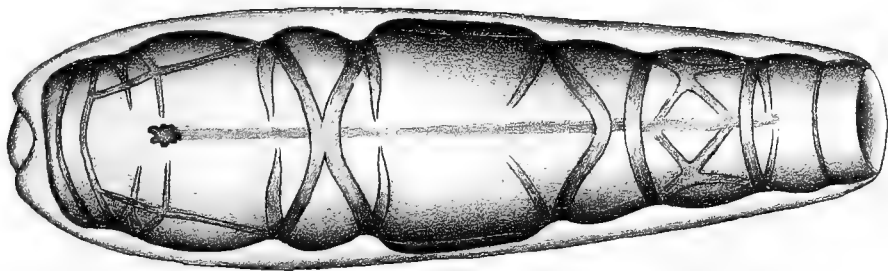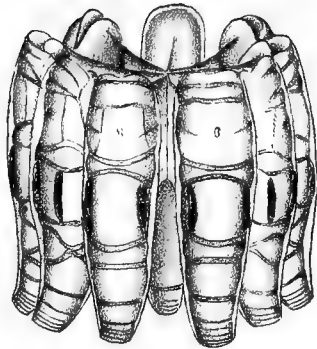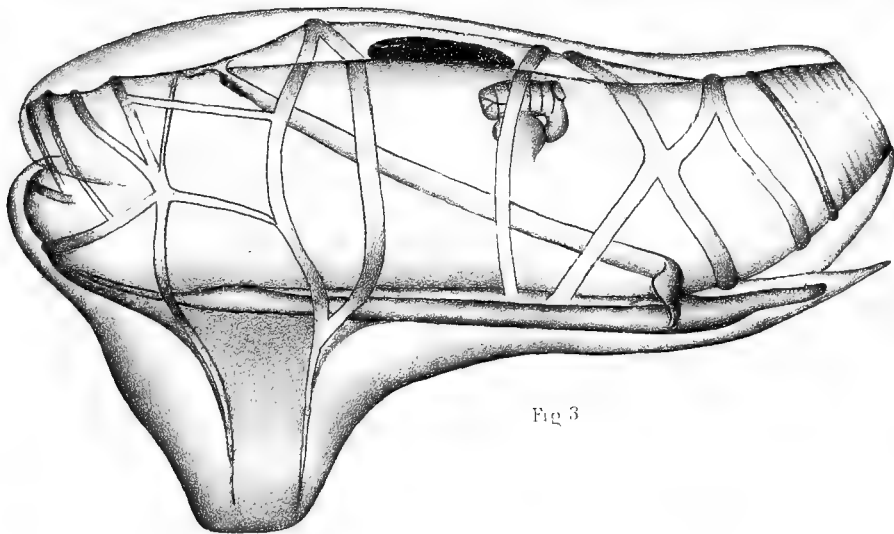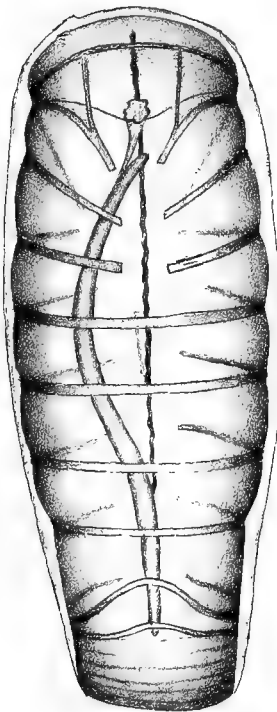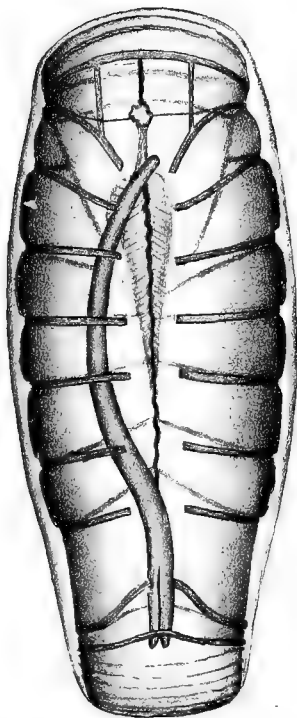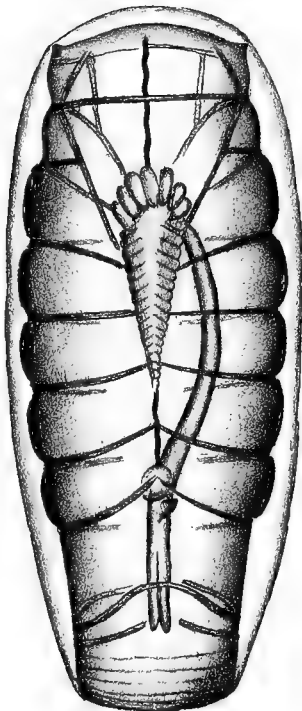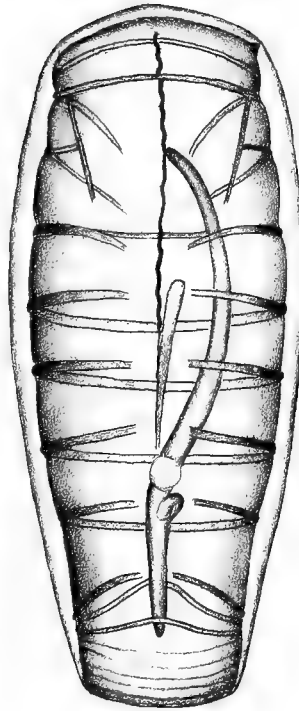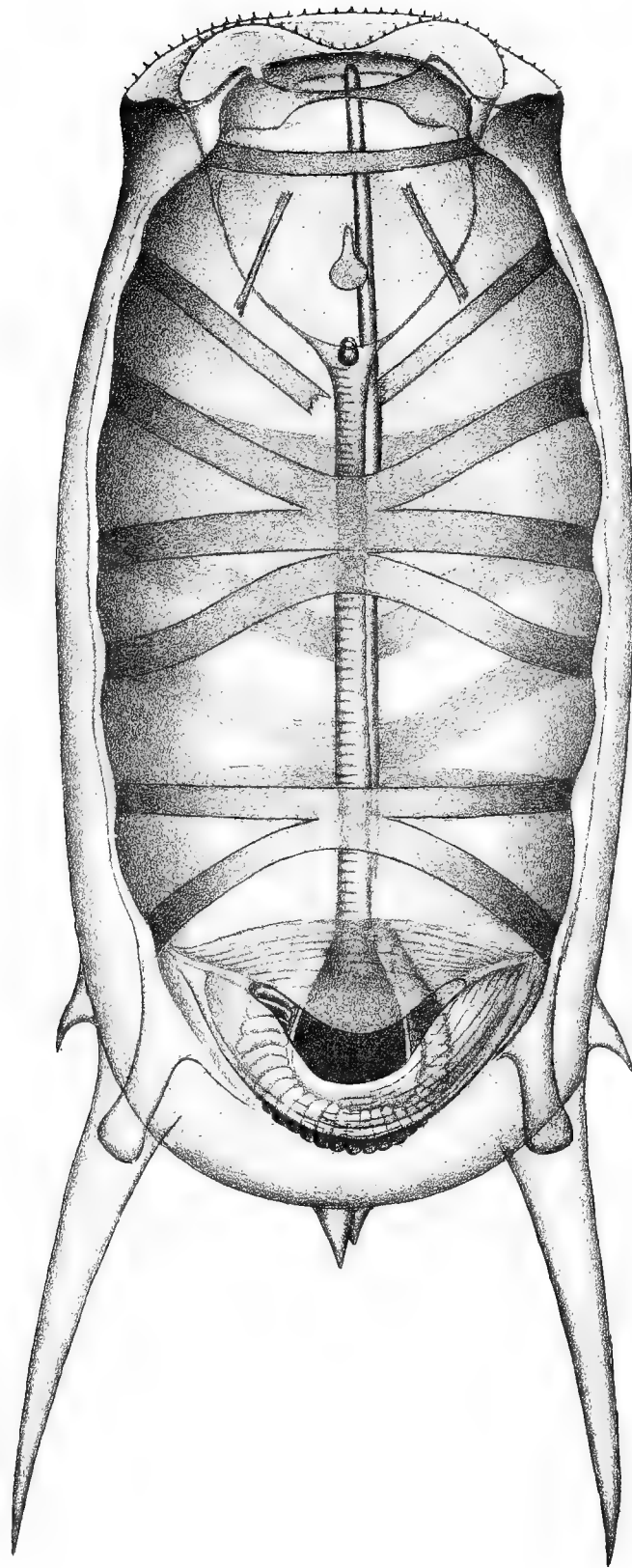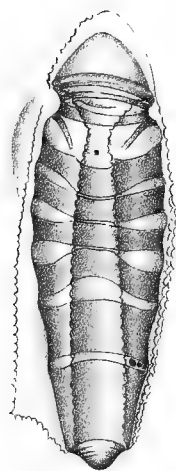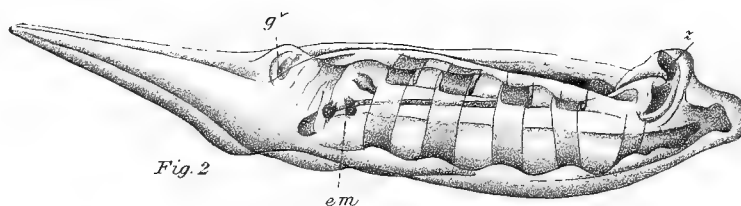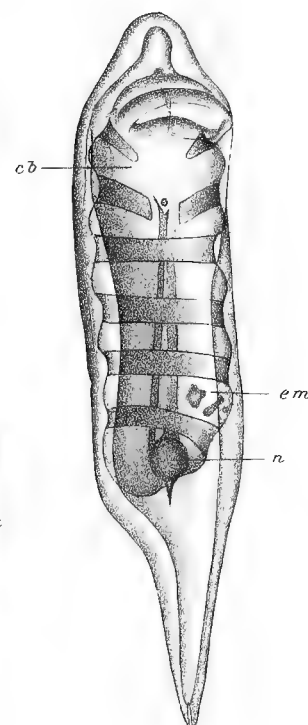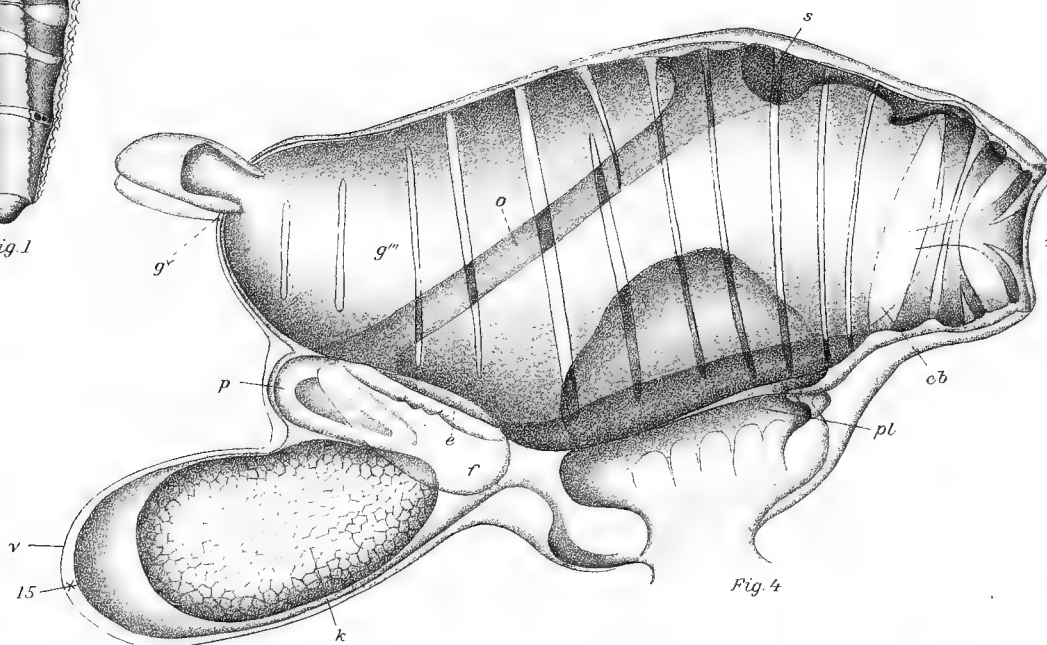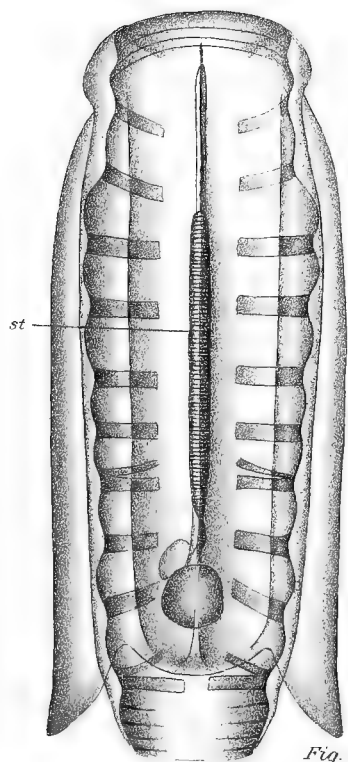
Fig. 1
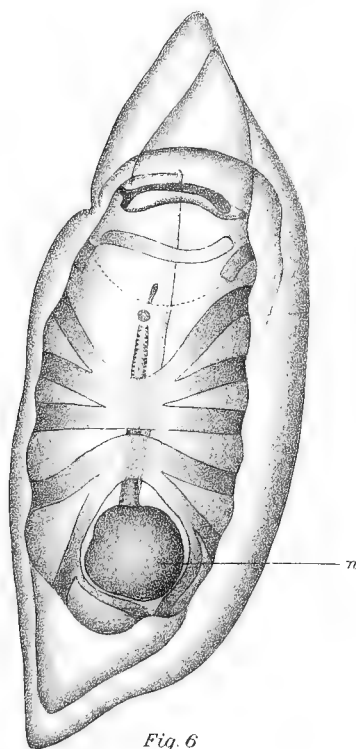
Fig. 2
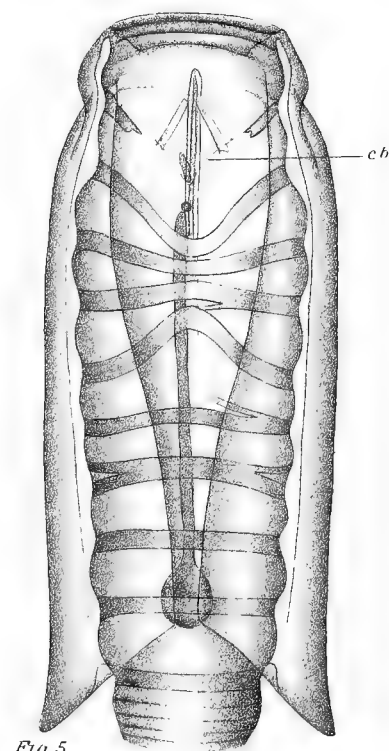
Fig. 3
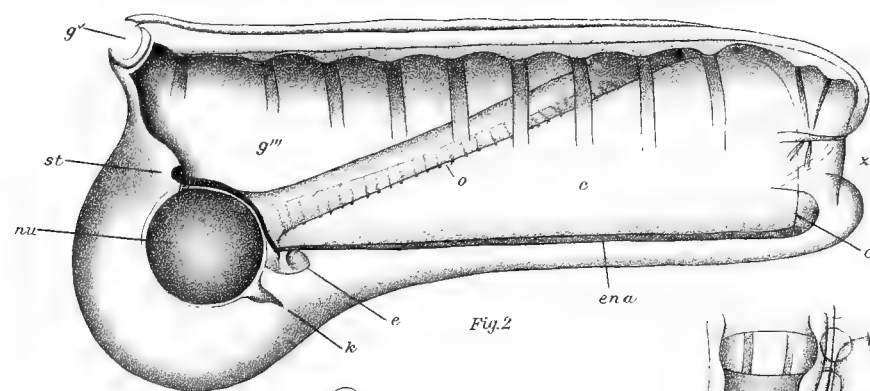
Fig. 4
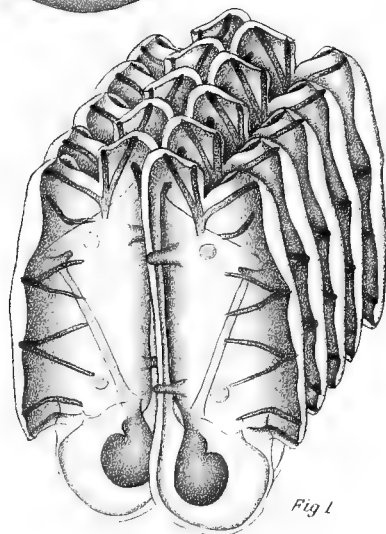
Fig. 5
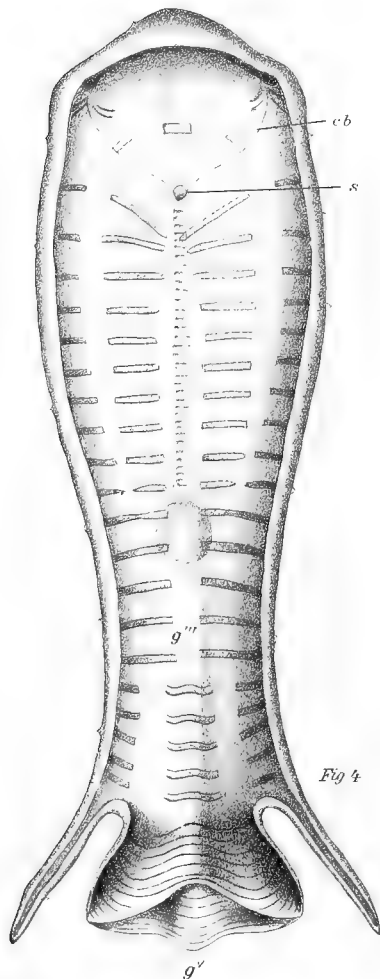
Fig. 6
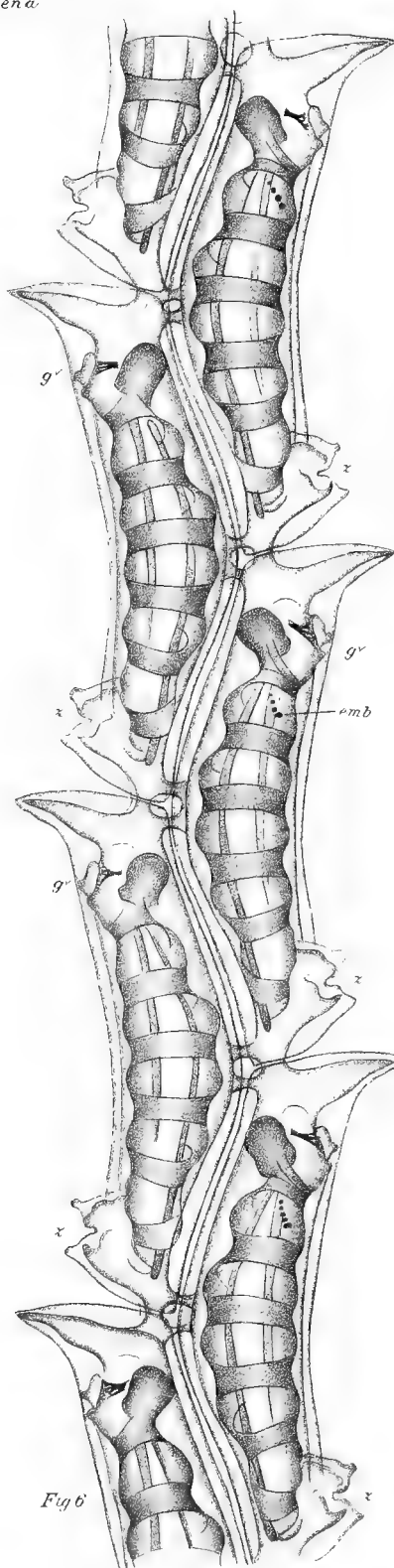
Fig. 7

Fig.2

Fig 1
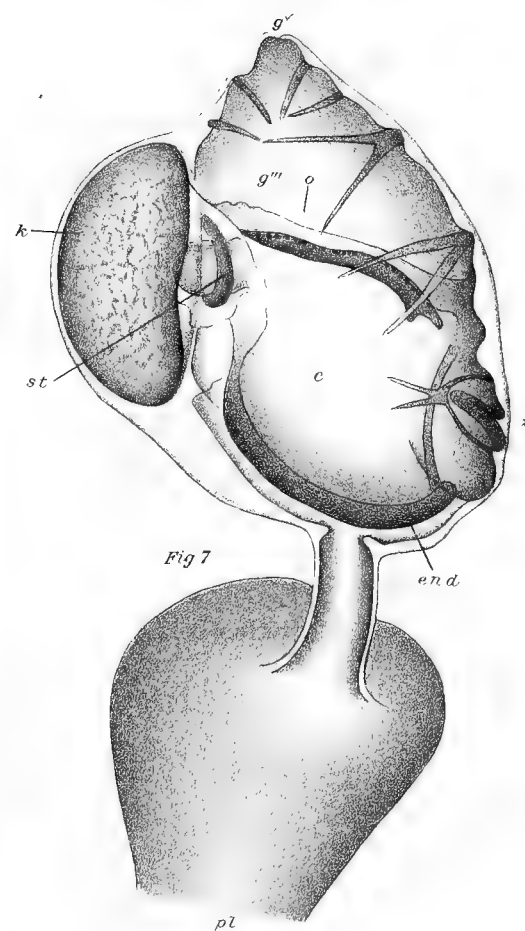
Fig 4
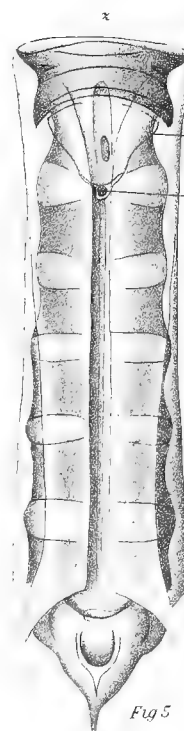
Fig 6

Fig 7

Fig 5
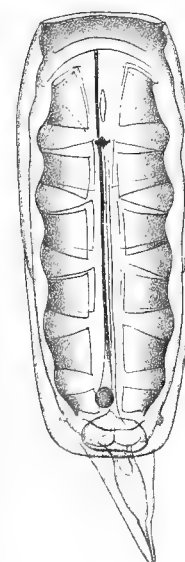
Fig 3
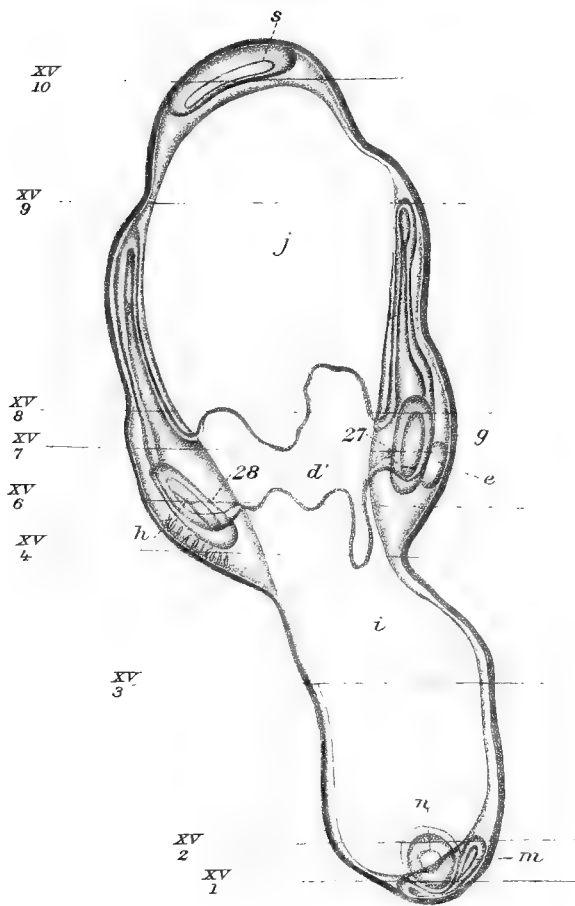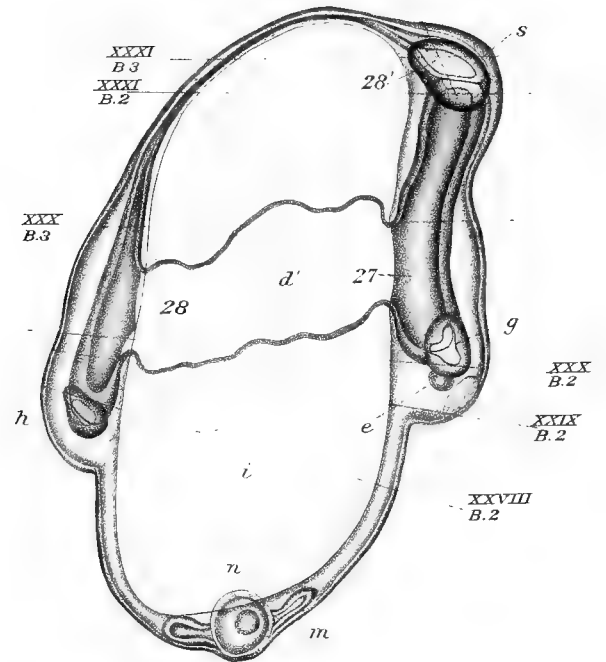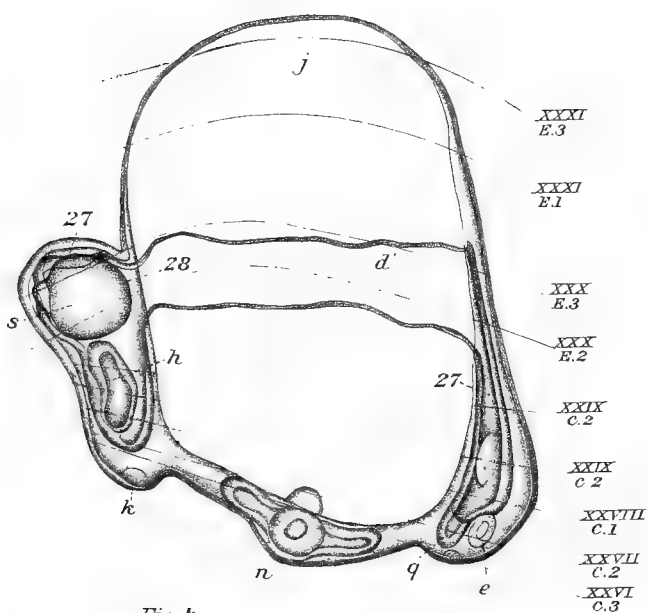
PLATE V
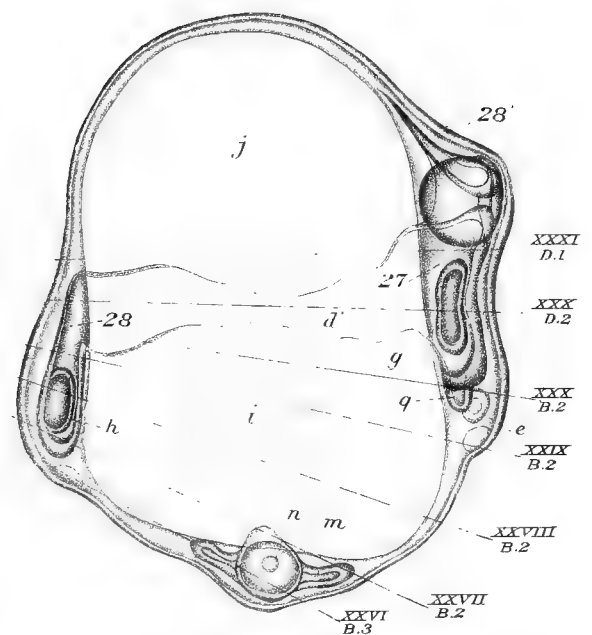
Fig. 1

Fig. 2

Fig. 4

Fig 3
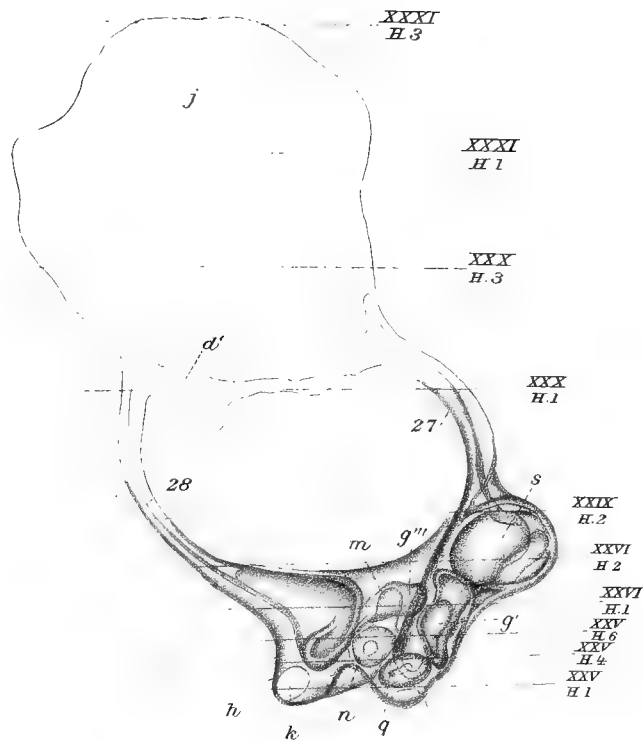
Fig.1

Fig.3
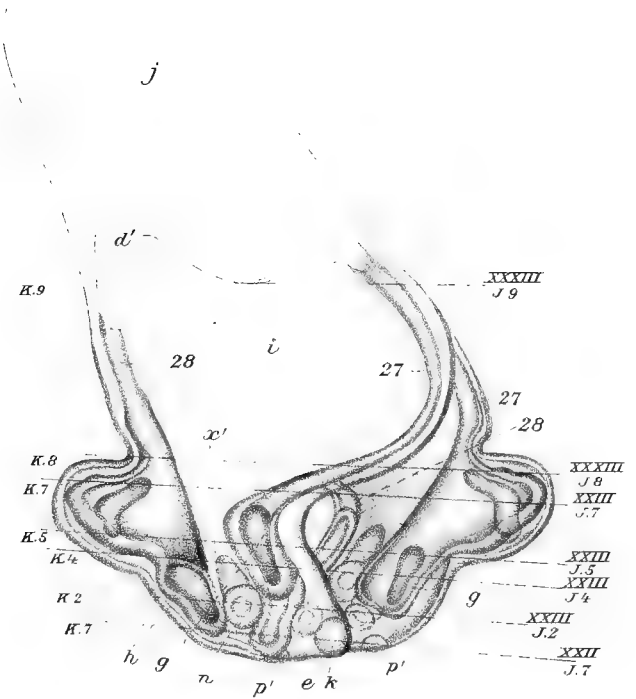
Fig.2
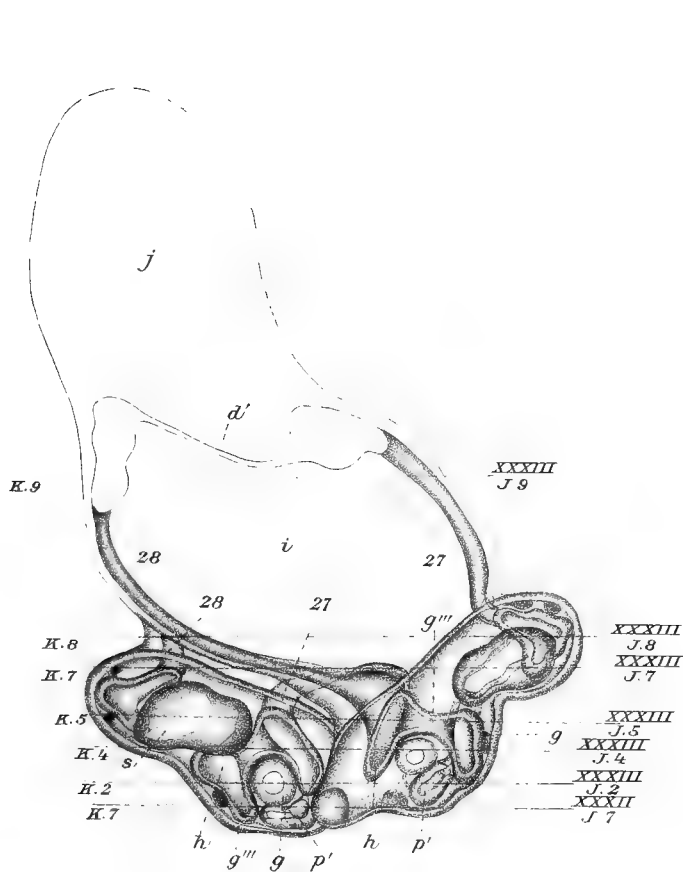
Fig.4
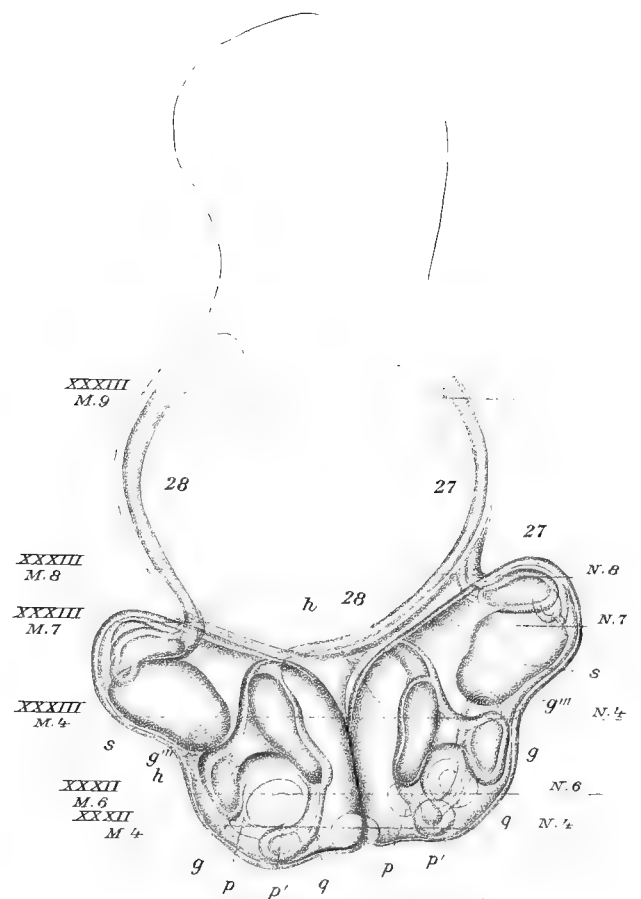
Fig.3

Fig.4

Fig.2

Fig.1

Fig.5

PLATE VIII
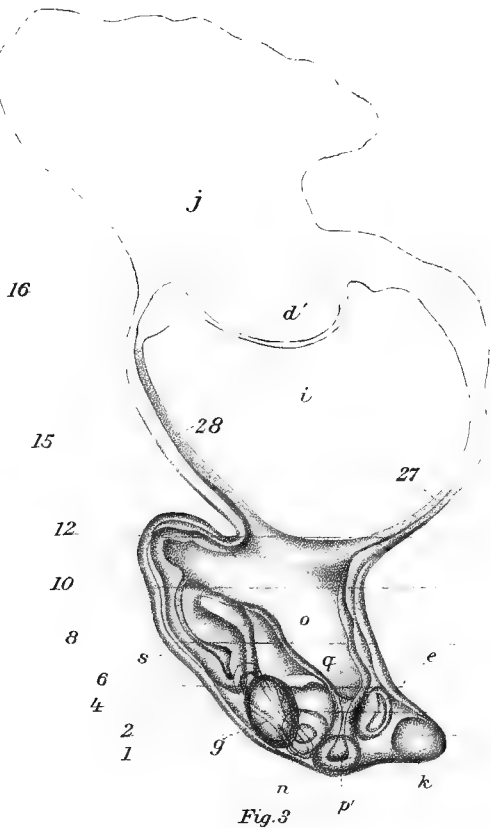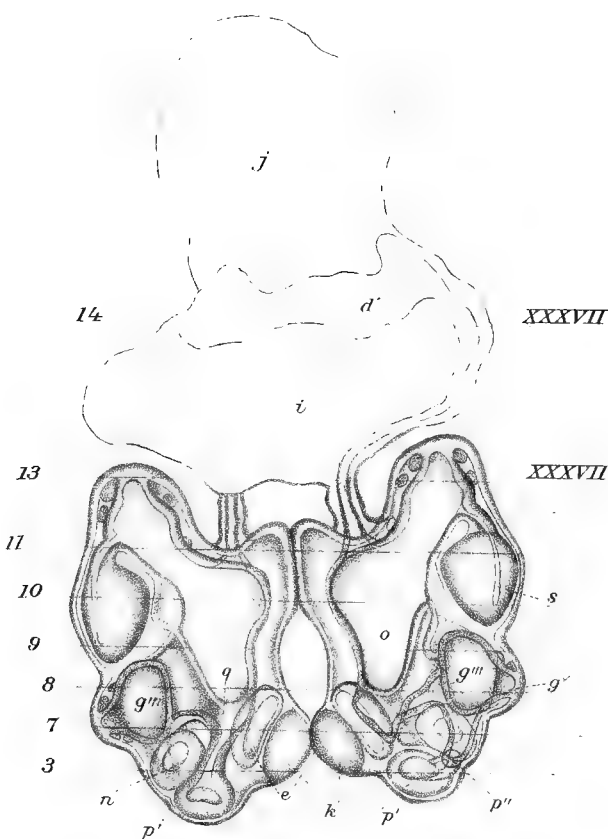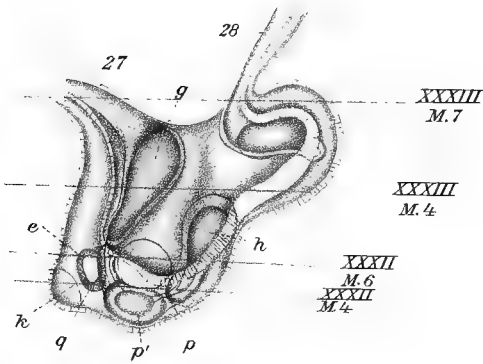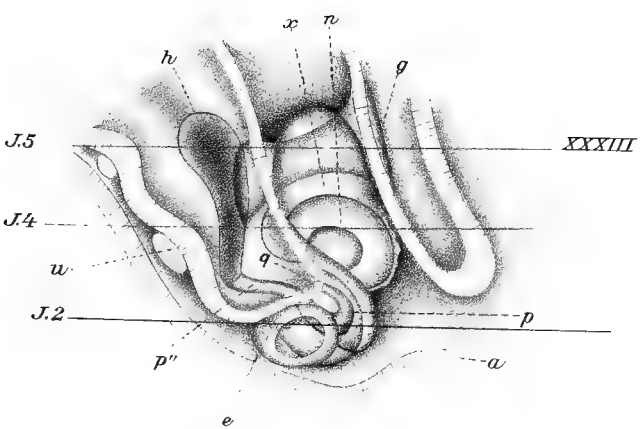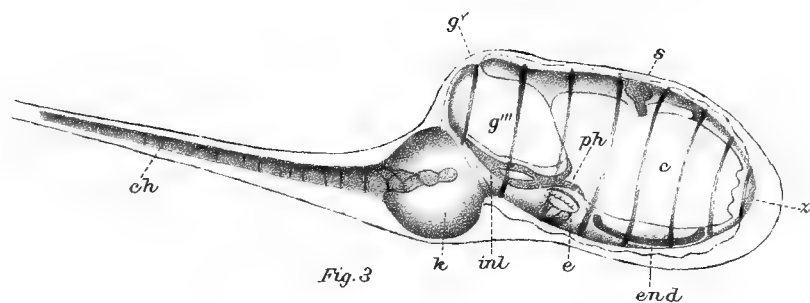
Fig.3

Fig.4

Fig.2

Fig.5

XXXVIII

Fig.1

Fig.6

PLATE IX
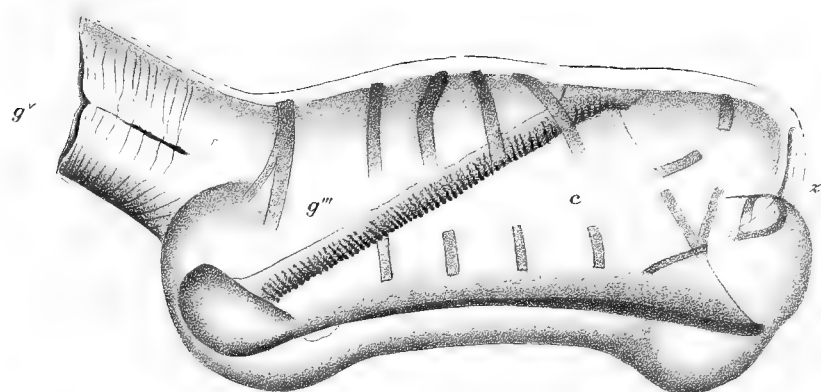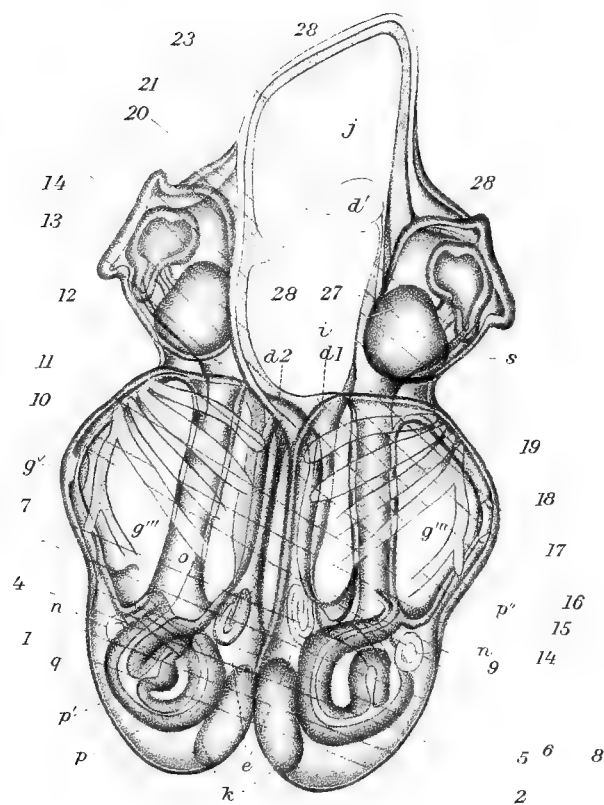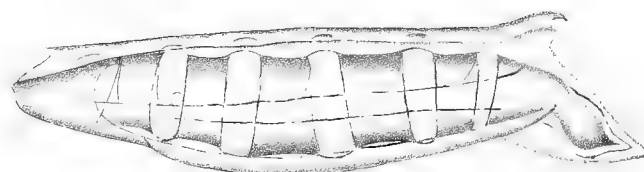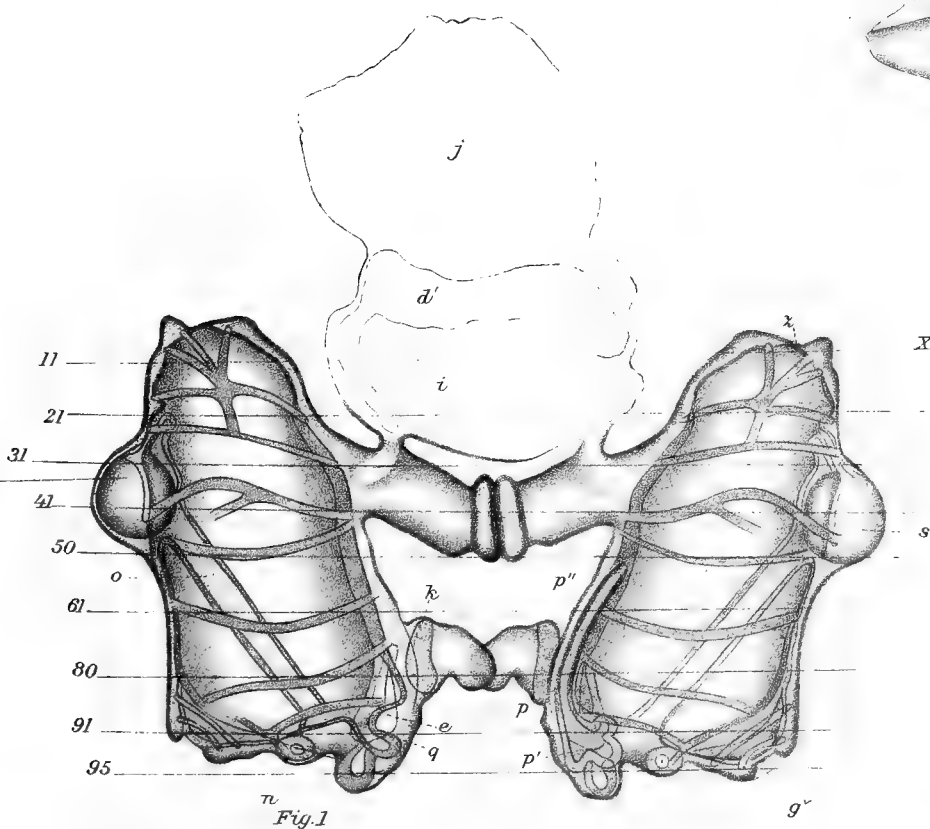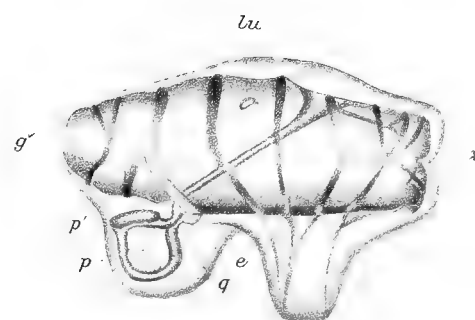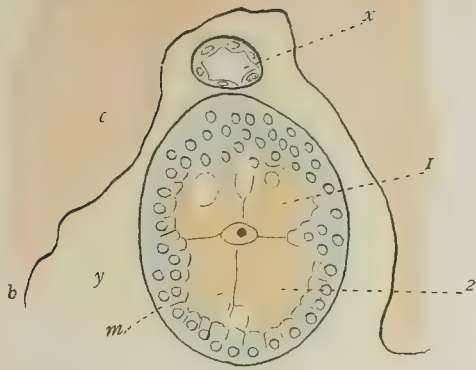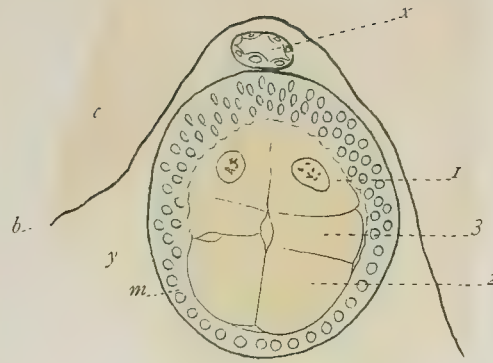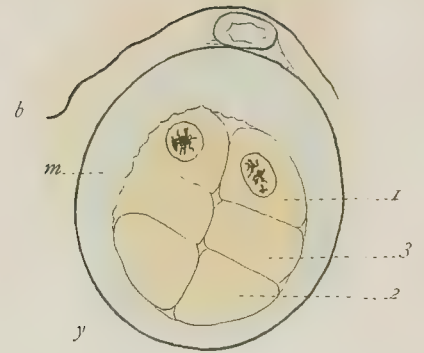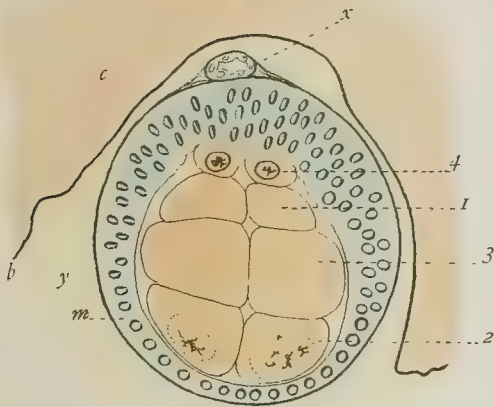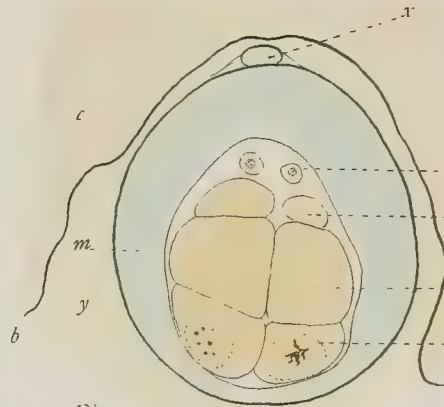
Fig. 1
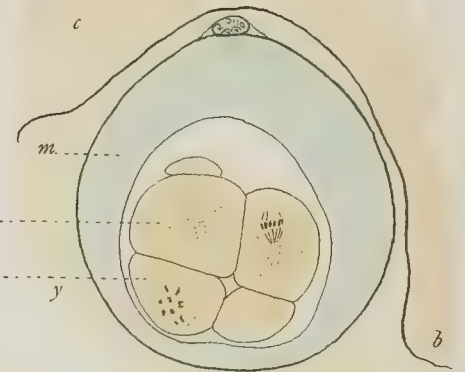
Fig. 2
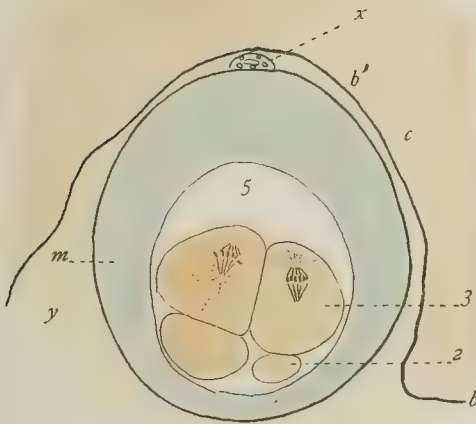
Fig. 3
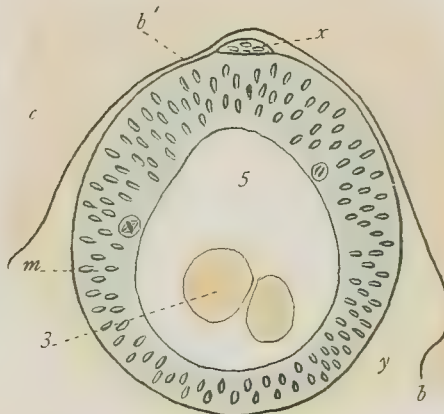
Fig. 4
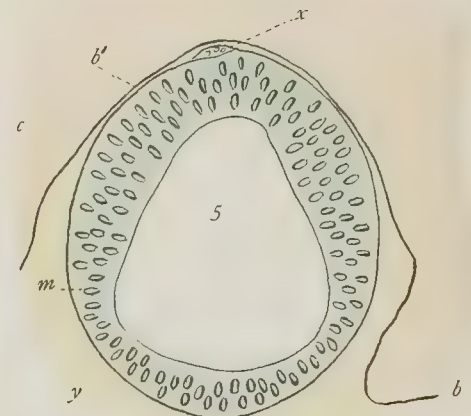
Fig. 5

Fig. 6

Fig. 7

Fig. 8

Fig. 9

Fig. 10

Fig. 11

Fig. 12

PLATE X

Fig. 4

Fig. 2

Fig. 3
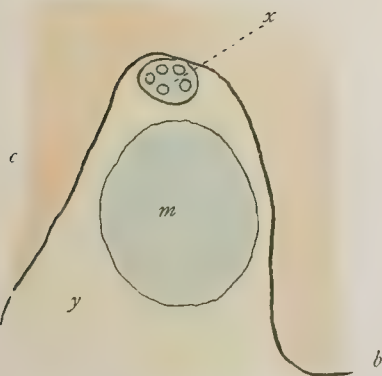
Fig. 1
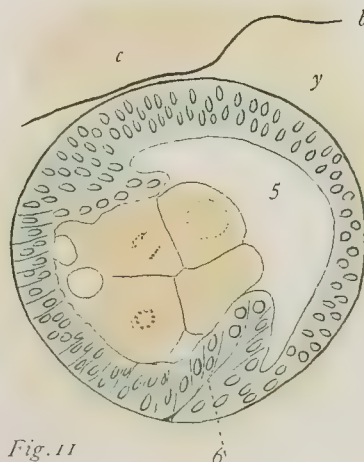
Fig. 5
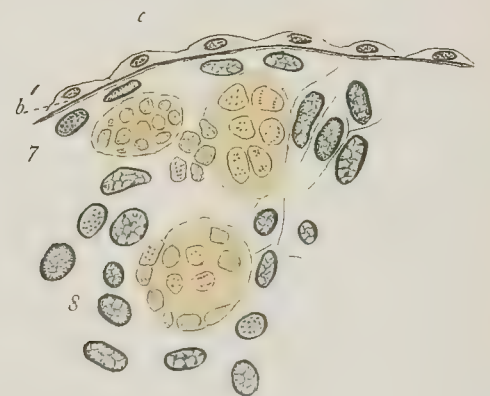
Fig. 6

Fig. 9
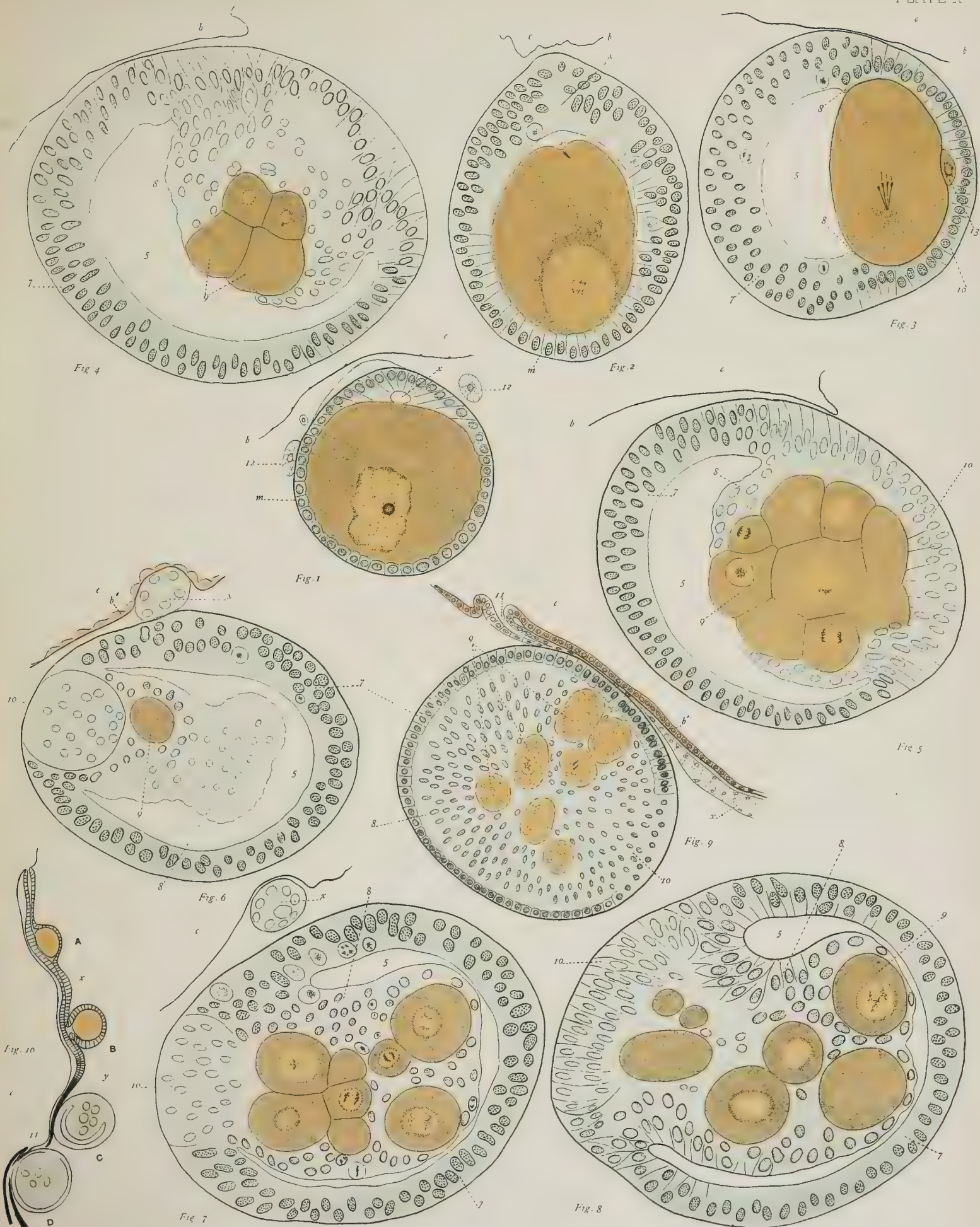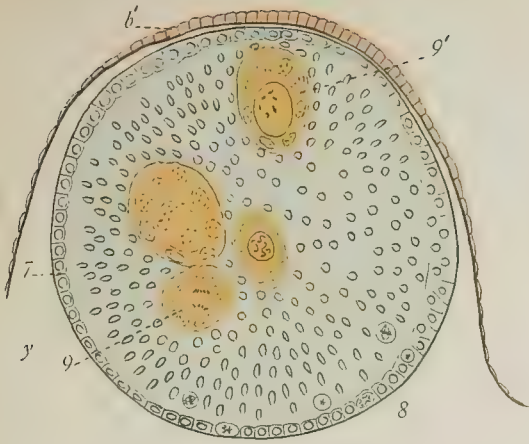
Fig. 10
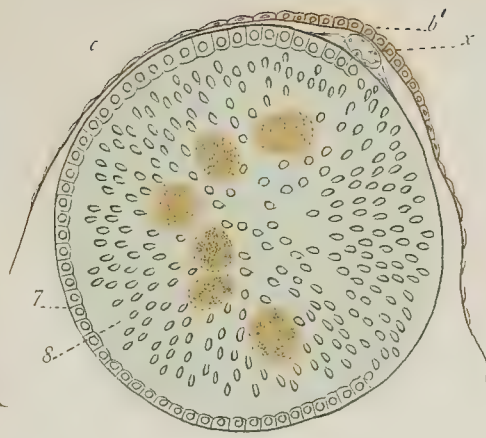
Fig. 7
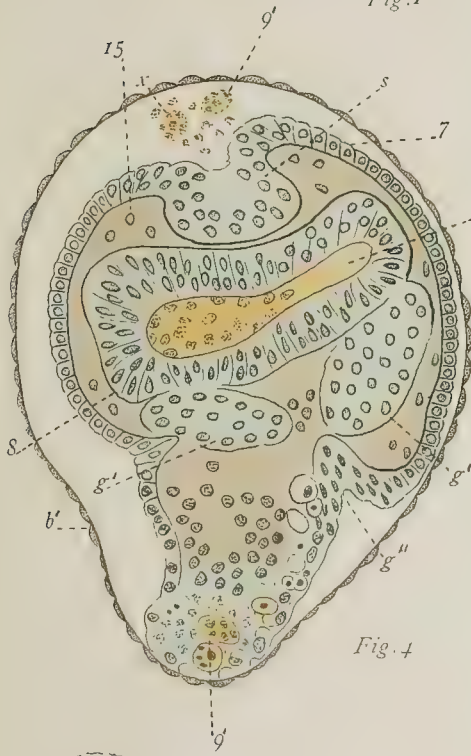
Fig. 8

Fig.1

Fig.2

Fig.3

Fig.4

Fig.5

Fig.6

Fig.9

Fig.10

Fig.11

Fig.7

Fig.8

Fig.12

PLATE XII

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5
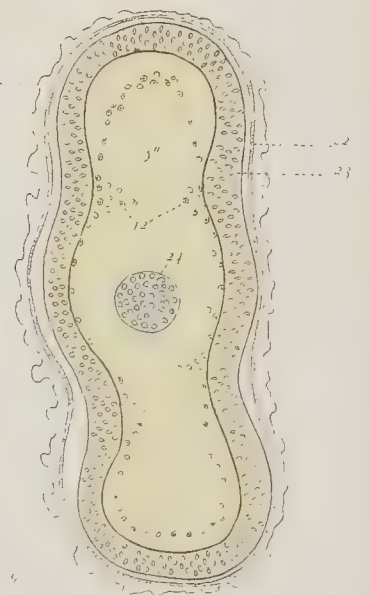
Fig. 6

Fig. 10
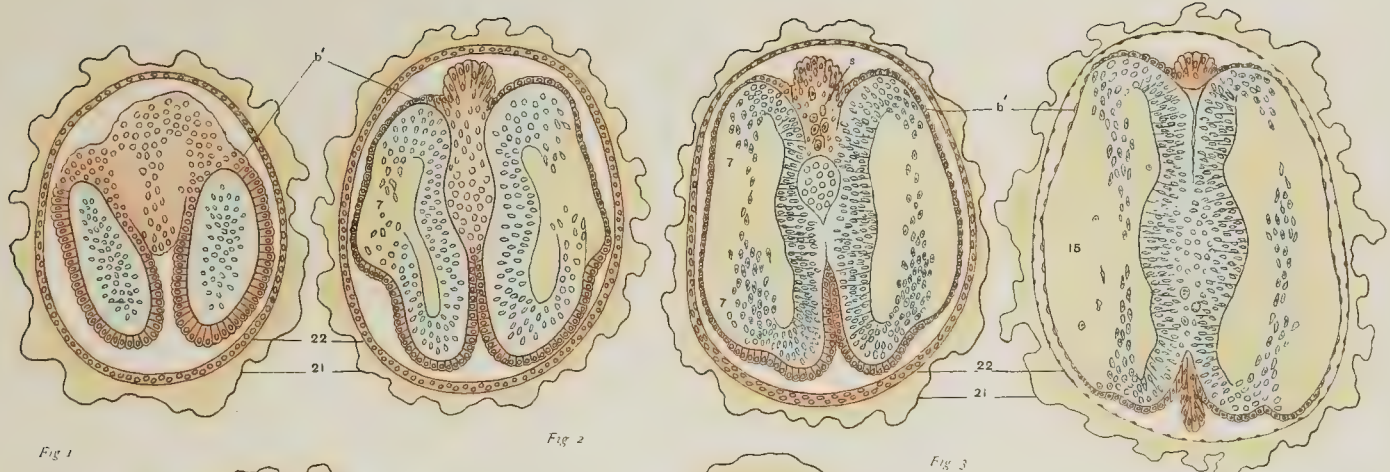
Fig. 7

Fig. 8

Fig. 9

Fig 1

Fig 2

Fig 3

Fig 4

Fig 5

Fig 6

Fig 8

Fig 9

Fig 7
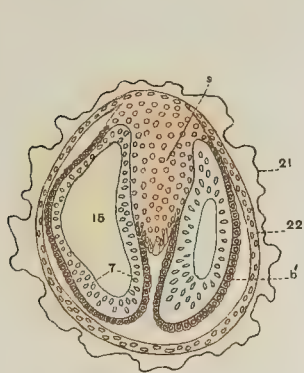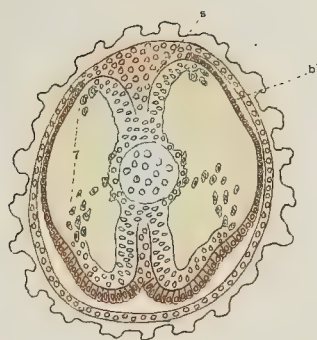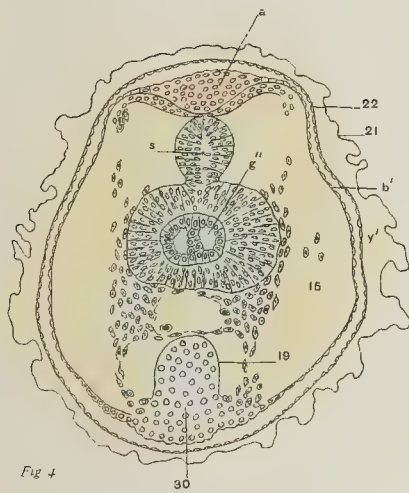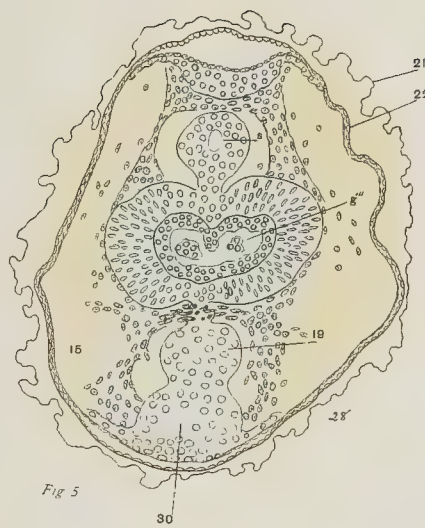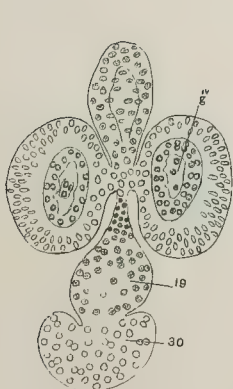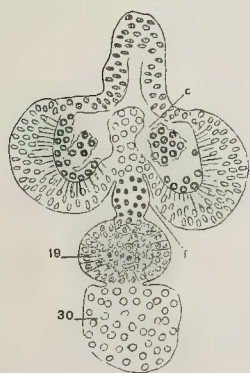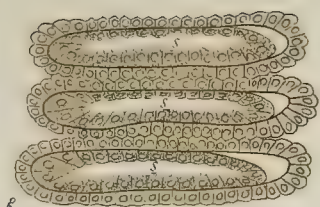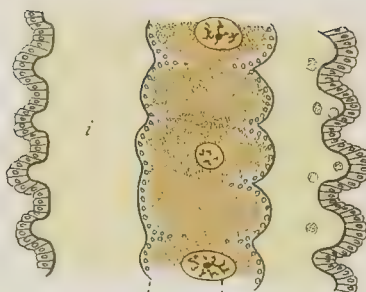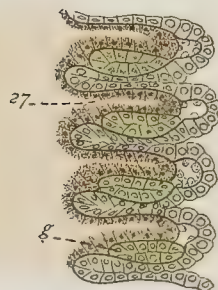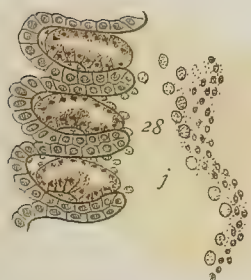
PLATE XIV.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6

Fig. 7

Fig. 8

Fig. 9

PLATE XV

Fig. 10

Fig. 3

Fig. 9

Fig. 2

Fig. 1

Fig. 8

Fig. 7

Fig. 6

Fig. 5

Fig. 11

Fig. 4

Fig. 12

Fig.1

Fig.4

Fig.2

Fig.6

Fig.7

Fig.3

25
22
21
y'

25
21
22
y'

PLATE XVII

SALPA.

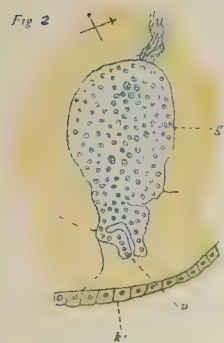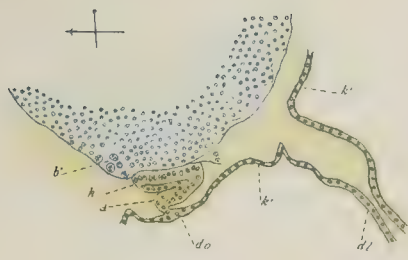Fig 1

Fig 5

Fig 6

Fig 7

Fig 2

Fig 3

Fig 4

W.K.BROOKS, autograph

PLATE XVIII.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6

Fig. 7

Fig. 8

PLATE XIX.
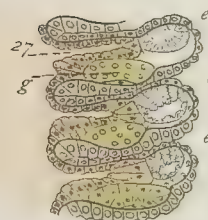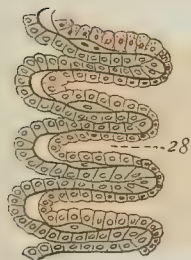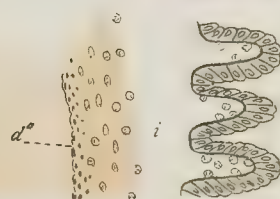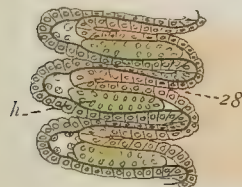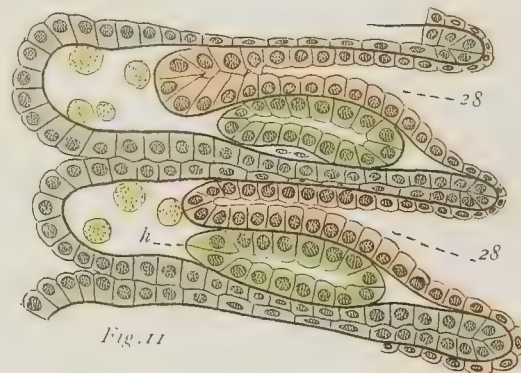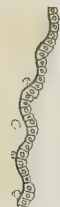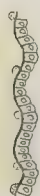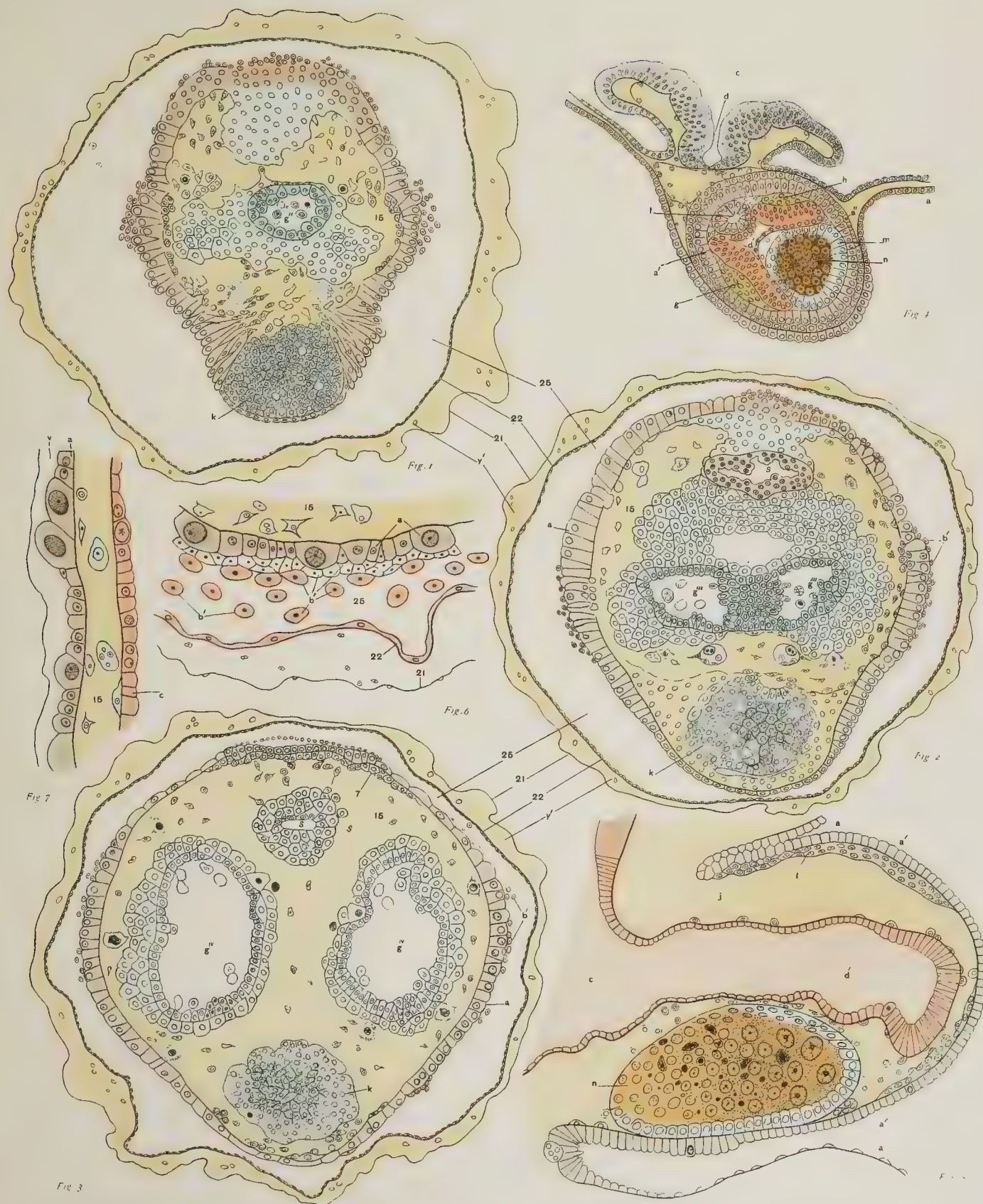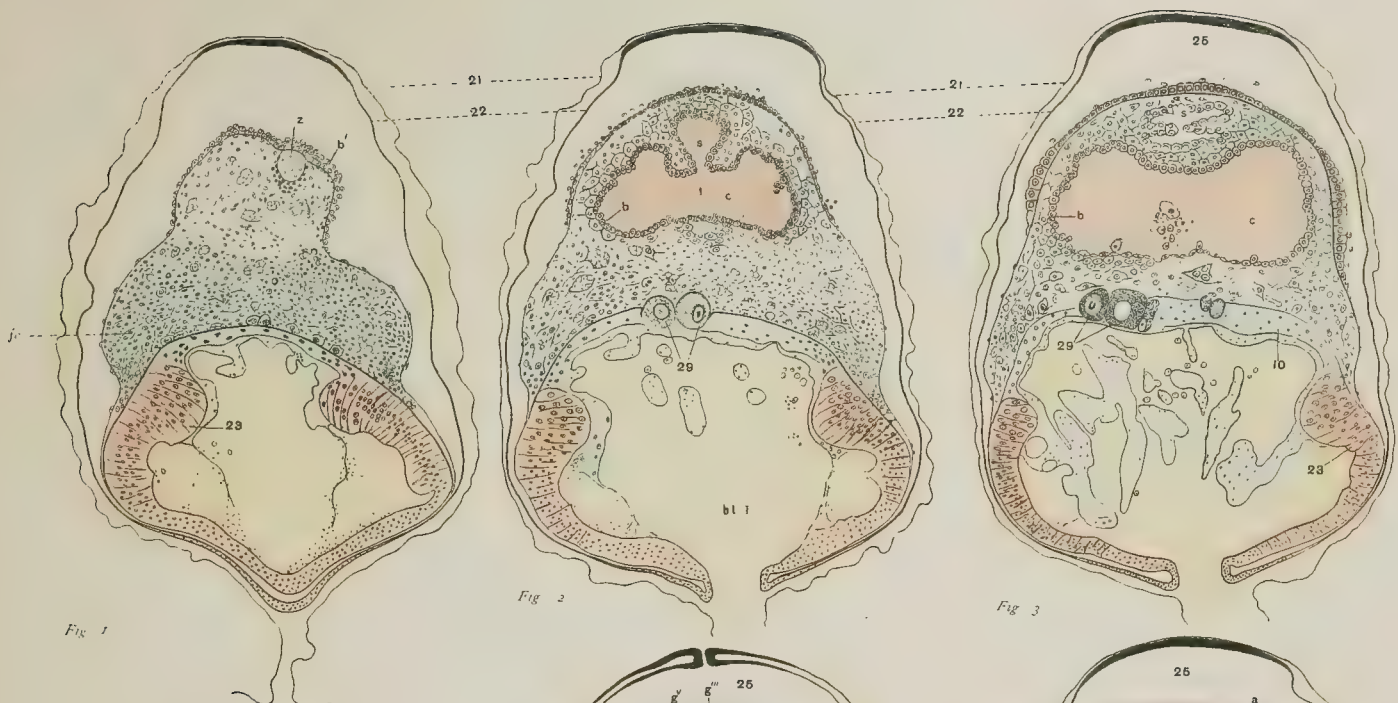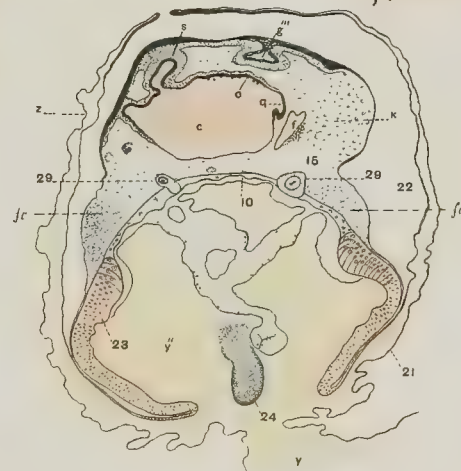
SALPA.
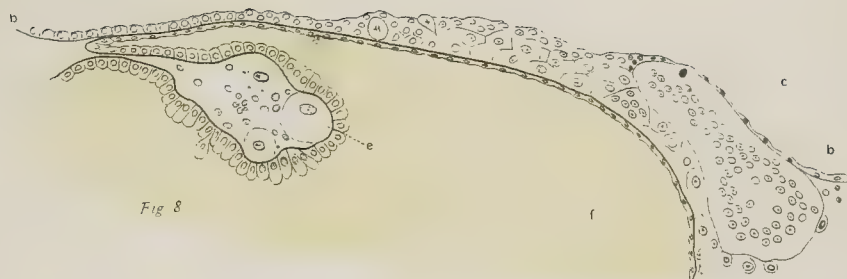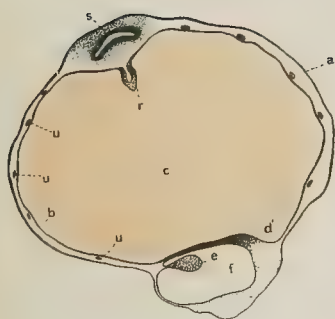
Fig 1
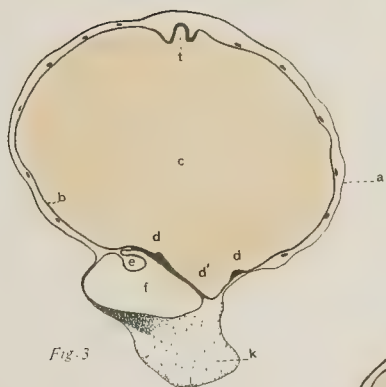
Fig 8

Fig 7

Fig.9

Fig 2

Fig. 3

Fig 6

Fig. 10

Fig.4

Fig 5
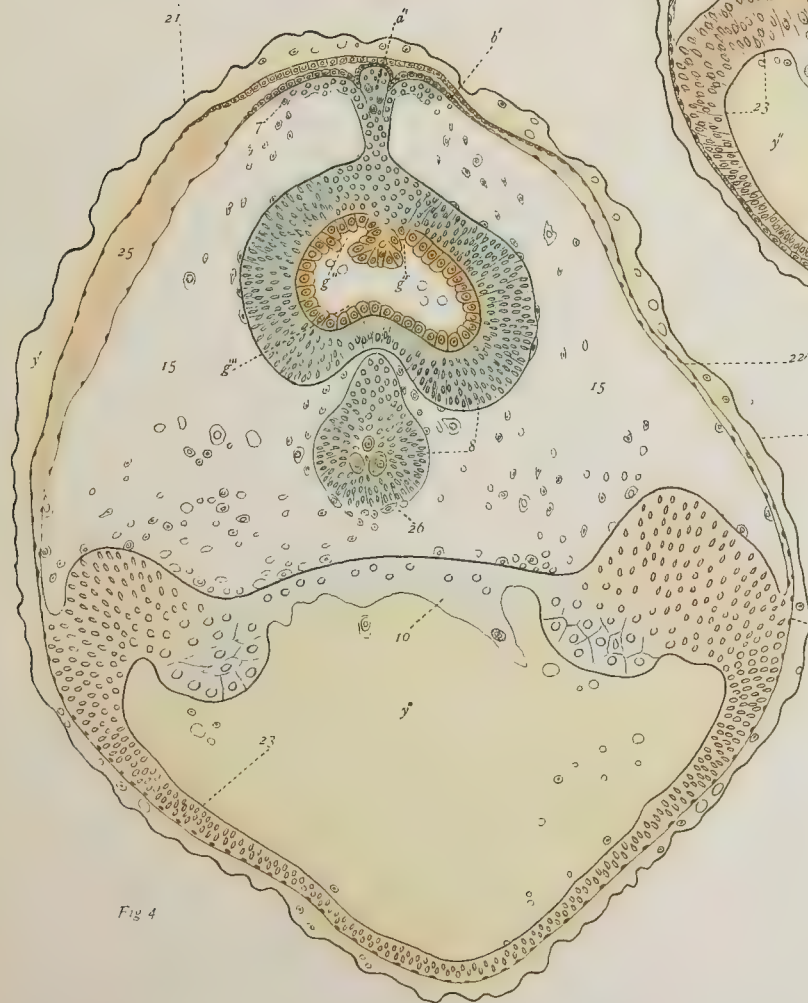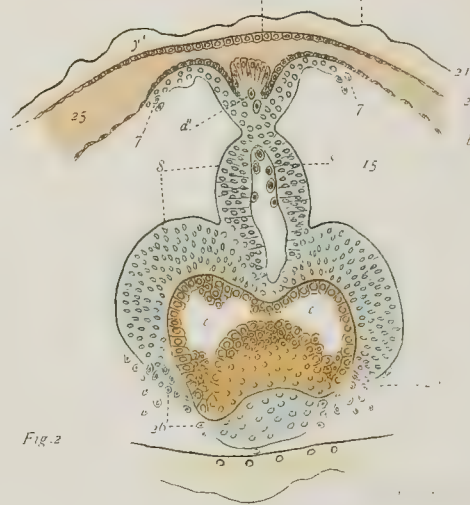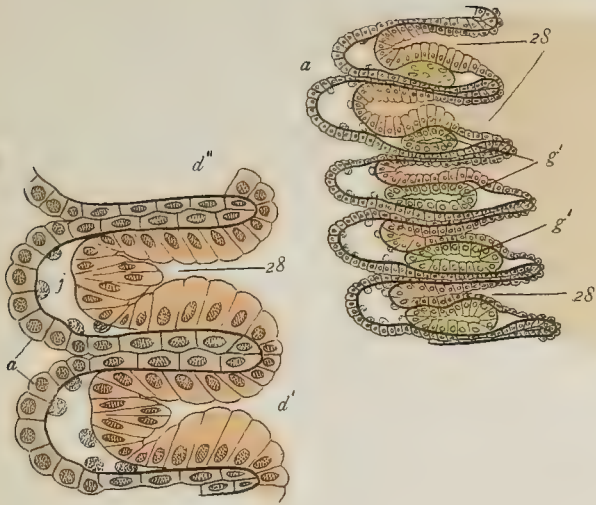
W.K.BROOKS del et grauph

PLATE XX

Fig. 2

Fig. 1'

Fig. 3

Fig. 5

Fig. 4

Fig. 7

Fig. 6.

PLATE XXI

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6

Fig. 7

PLATE XXII

SALPA.

Fig.5

Fig.4

Fig.3

Fig.1

Fig.2

PLATE XXIII

SALPA.

Fig.4

Fig.10

Fig 9

Fig.8

Fig 3

Fig.7

Fig.2

Fig 5

Fig.1

Fig.6

PLATE XXIV

Fig. 5

Fig. 4

Fig. 3

Fig. 2

Fig. 1
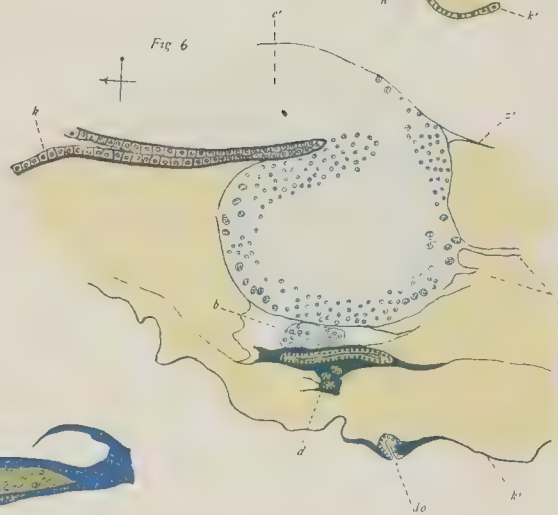
PLATE XXV

SALPA.

Fig. 4

Fig. 7

Fig. 3

Fig. 2

Fig. 6

Fig. 1

Fig. 5

PLATE XXVI.

Fig. 3

Fig. 2

Fig. 1

Fig. 3

Fig. 2

Fig. 1

PLATE XXVIII.

Fig. 2

Fig. 1

Fig. 6

Fig. 2

Fig. 5

Fig. 8

Fig. 7

Fig. 4

Fig. 1

Fig. 3

PLATE XXX

H'

G'

F'

E'

D'

C'

B'

j

H

G

F

E

D

C

B

27

d'

Fig. 3

H'

G'

F'

E'

D'

C'

B'

28

28

28

28

28

j

s

s

27

27

H

G

F

E

D

C

B

d'

s

e

g

Fig. 2

H'

G'

F'

E'

D'

C'

B'

A'

28

28

28

28

28

s

h

27

27

27

s

e

g

H

G

F

E

D

C

B

i

d'

d''

Fig. 1

PLATE XXXI.
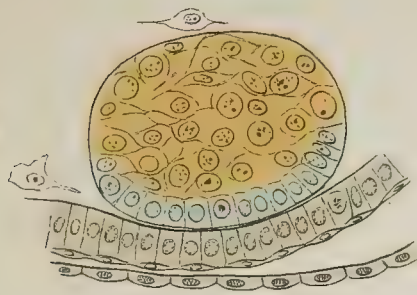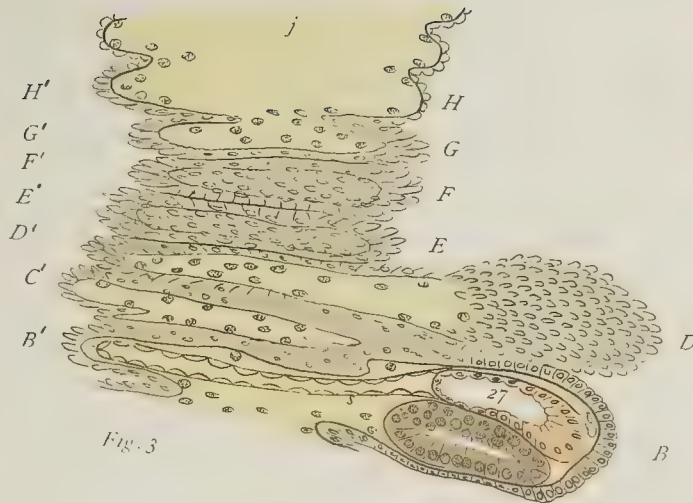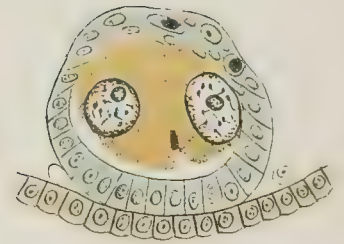
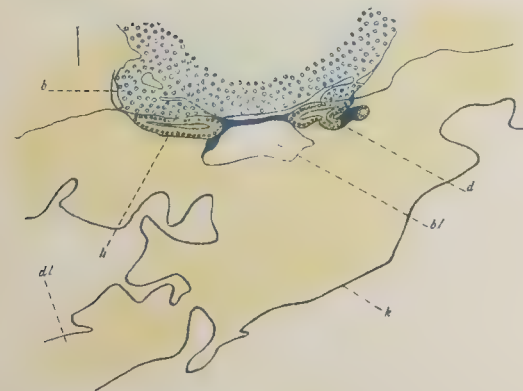Fig. 4

Fig. 3

Fig. 7

Fig. 5

Fig. 2

Fig. 8

Fig. 6

Fig. 9

Fig. 1

PLATE XXXII.

Fig. 5

Fig. 4

Fig. 3

Fig. 2

Fig. 1

Fig. 8

Fig. 7

Fig. 6

Fig 4

Fig 5

Fig 6

Fig 7

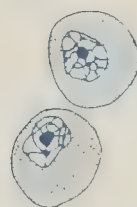Fig 8

Fig 9

Fig 10

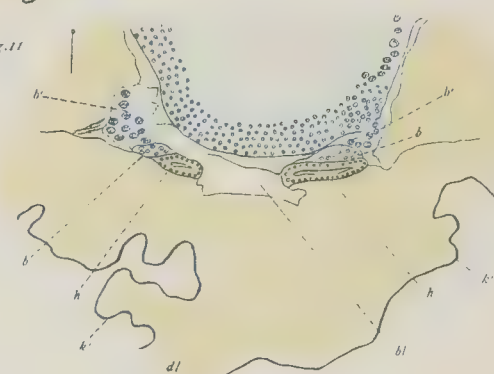Fig 1

Fig 2

Fig 3

PLATE XXXV

PLATE XXXVI.

Fig. 9

Fig. 8

Fig. 7

Fig. 6

Fig. 5

Fig. 3

Fig. 4

Fig. 2

Fig. 1

Fig. 16

Fig. 15

Fig. 11

Fig. 13

Fig. 12

Fig. 11

Fig. 10

Fig. 14

Fig. 13

Fig. 12

Fig. 11

Fig. 10

Fig. 9
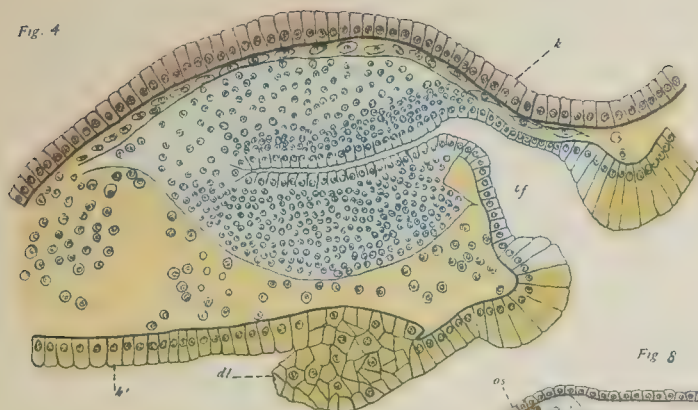
Fig. 8

Fig. 7

Fig. 6

Fig. 5

Fig. 4

Fig. 3

Fig. 2

Fig. 1

Fig. 24

Fig. 23

Fig. 22

Fig. 21

Fig. 20

Fig. 19

Fig. 18

Fig. 17

Fig. 16

Fig. 15

Fig. 25

Fig. 26

PLATE XXXIX.

Fig. 7
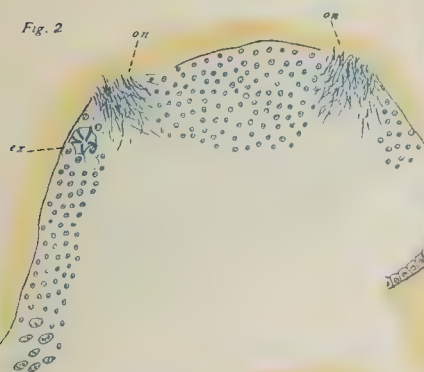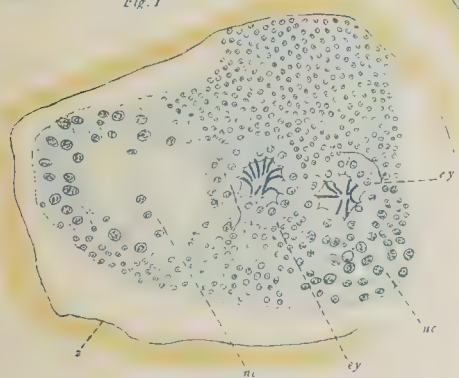
Fig. 1

Fig. 9

Fig. 8

Fig. 2

Fig. 10

Fig. 3

Fig. 11

Fig. 4

Fig. 5

Fig. 12

Fig. 6

PLATE XI.

Fig.18

Fig 13

Fig.19

Fig.14

Fig 20

Fig 15

Fig.16

Fig 21

Fig.22

Fig 23

Fig 17

PLATE XLI.
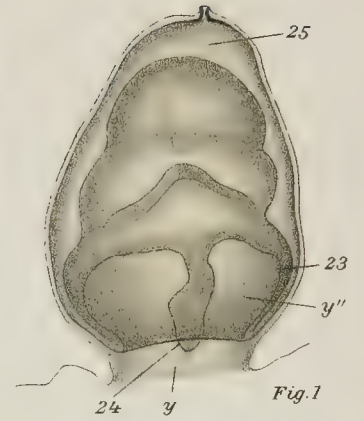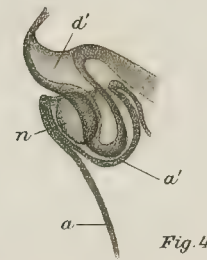
Fig. 2.

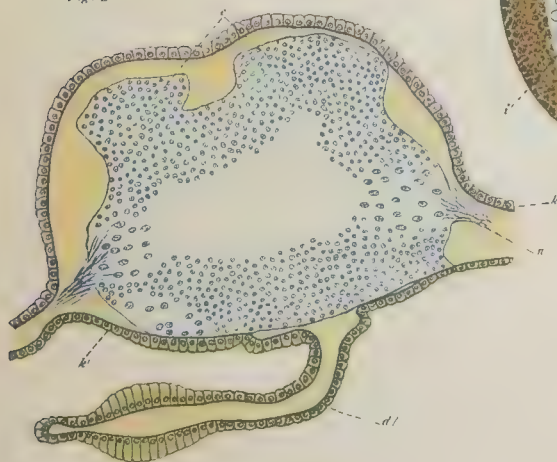Fig. 10.

Fig. 1.

Fig. 4.

Fig. 6.

Fig. 5.

Fig. 3.

Fig. 7.

Fig. 9.

Fig. 8.

PLATE XLII
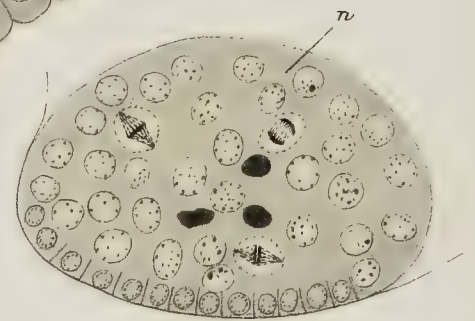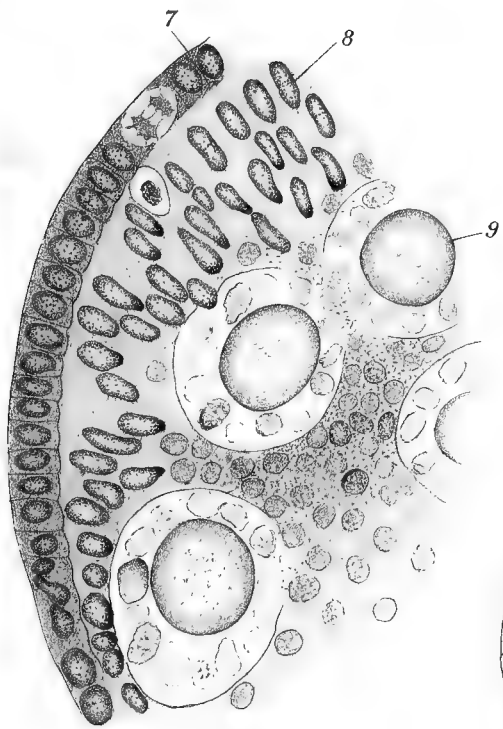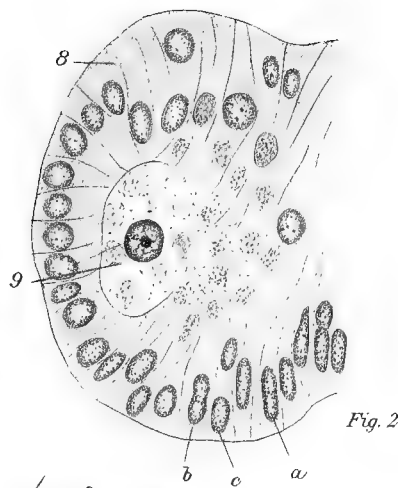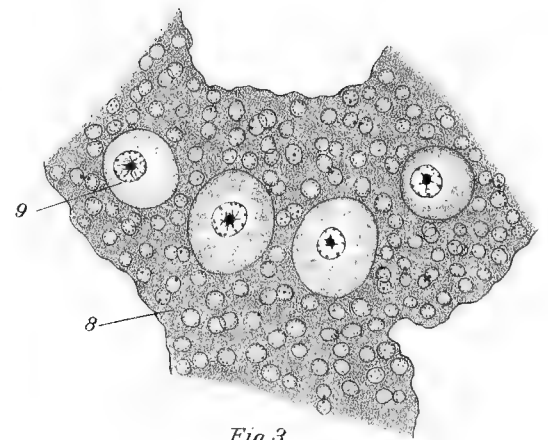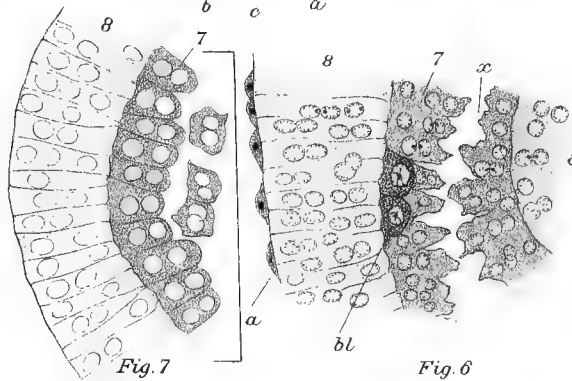
Fig.1

Fig. 2

Fig. 3

Fig. 7

Fig. 6
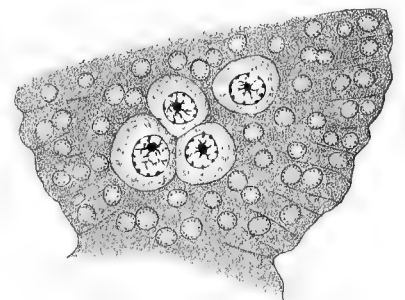
Fig. 4
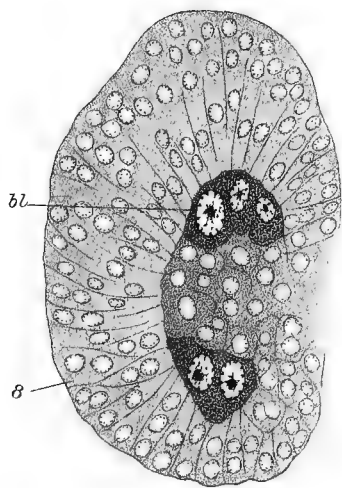
Fig. 5
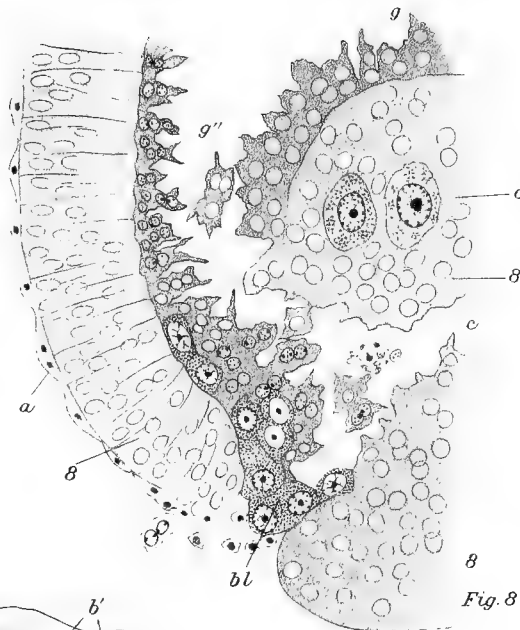
Fig. 8
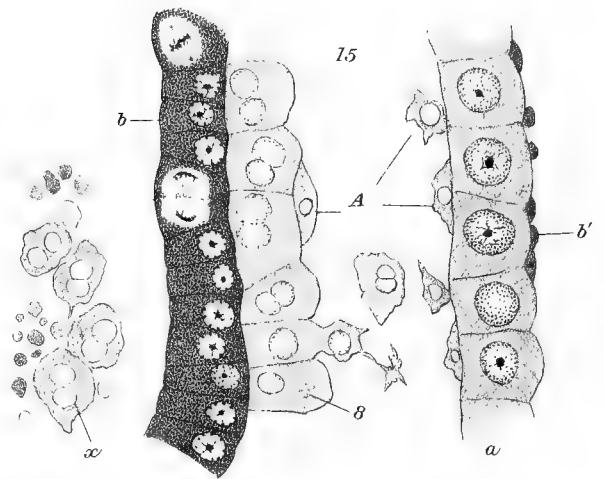
Fig. 9
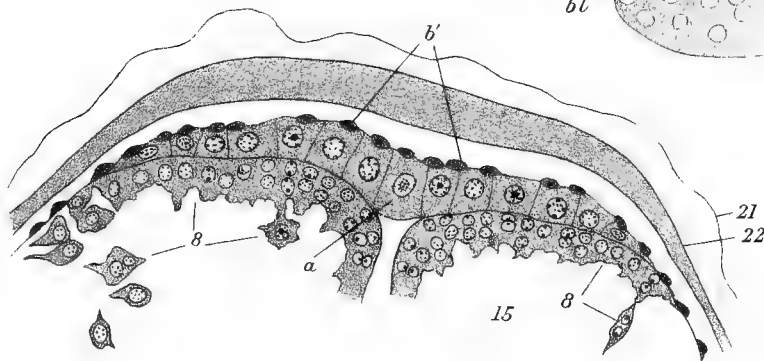
Fig. 11

Fig. 10

Fig. 1.

Fig 2.

Fig 1

Fig 2

Fig. 5.

Fig. 4.

Fig 3.

PLATE XLIV

SALPA.

Fig 1

Fig 2

Fig 3

Fig 4

Fig 5

Fig 6

Fig 7

Fig 8

PLATE XLVI.

Fig 1

Fig 3.

Fig. 2.

PLATE XLVIII.
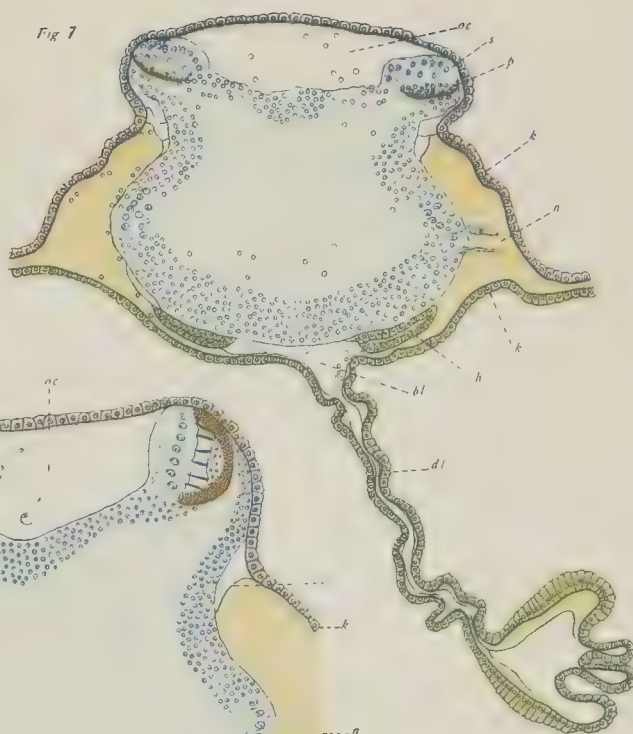
PLATE XLIX.

SALPA.

Fig.1

Fig.9

Fig.2

Fig.7

Fig.8

Fig.3

Fig.4

Fig.5

Fig.6

PLATE XLIX.

Fig. 2

Fig. 3

Fig. 1

Fig. 4

Fig. 6

Fig. 5

Fig. 7

Fig. 8

Fig. 9

Fig. 10

Fig. 12

Fig. 11

PLATE LI.

PLATE LII.

Fig 3

Fig 4

Fig.5

Fig 6

Fig 2

Fig 1

Fig 9

Fig 8

Fig 7

PLATE LIV.

PLATE LV.

PLATE LV.

PLATE LVI

PLATE LVII